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Sir:

Transmitted herewith for filing is the patent application of:

APPLICANTS: **Jeffrey D. Rothstein, Mandy Jackson, Glen Lin, Robert Law, and Irina Orlov**

FOR: **GLUTAMATE TRANSPORTER ASSOCIATED PROTEINS AND METHODS OF USE THEREOF**

Enclosed are the following papers, including all those required to receive a filing date under 37 CFR § 1.53(b):

	Number of Pages
Specification	73
Claims	12
Abstract	1
Declaration	4 (Unsigned)
Drawings	26 sheets (Formal)

Enclosures:

- Declaration – Unsigned (4 pages)
- Power of Attorney – Unsigned (2 pages)
- Statement Under 37 C.F.R. § 1.821(f) (1 page)
- Verified Statement Under 37 C.F.R. § 1.821(f) (1 page)
- Paper Copy of the Sequence Listing (49 pages)
- Computer-Readable Form (CRF) Copy of Sequence Listing (1 diskette); and
- Return Postcard

This application claims priority under 35 U.S.C. § 119(e)(1) to U.S. Provisional Application Serial No. 60/161,007, filed October 23, 1999 and U.S. Provisional Application Serial No. 60/206,157, filed May 22, 2000, the contents of each of which are incorporated by reference in its entirety herein.

The filing fee is calculated as follows.

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Total Claims	**88** -20	=	68	X	$ 9.00	$ 18.00	=	$0	$0
Independent Claims	**22** -3	=	19	X	$ 39.00	$ 78.00	=	$0	$0
Multiple Dependent Claims Presented: _X_ Yes ____ No					$ 130.00	$ 260.00			$0
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If this application is found to be INCOMPLETE, of if a telephone conference would otherwise be helpful, please call the undersigned at (858) 677-1456.

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A duplicate copy of this letter is enclosed. Kindly acknowledge receipt of this application by returning the enclosed postcard.

Respectfully submitted,

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October 23, 2000
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Enclosures

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APPLICATION

for

UNITED STATES LETTERS PATENT

on

GLUTAMATE TRANSPORTER ASSOCIATED PROTEINS AND METHODS OF USE THEREOF

by

Jeffrey D. Rothstein

Mandy Jackson

Glen Lin

Robert Law

and

Irina Orlov

Sheets of Drawings: Twenty-Six (26)
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GLUTAMATE TRANSPORTER ASSOCIATED PROTEINS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e)(1) to U.S. Provisional Application Serial No. 60/161,007, filed October 23, 1999, and to U.S. Provisional Application Serial No. 60/206,157, filed on May 22, 2000, each herein incorporated by reference in their entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with Government support under NS33958 and NS70151, awarded, by the National Institutes of Health (NINDS). The Government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to protein-protein interactions and more specifically to Glutamate Transporter Associated Proteins involved in mediating glutamate transport, chloride transport and cytoskeletal stability and their association with glutamate transporter proteins.

BACKGROUND OF THE INVENTION

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system, acting on postsynaptic ionotropic glutamate receptors (particularly NMDA and AMPA receptors). In addition, glutamate stimulates a subset of metabotropic glutamate receptors (particularly the group I metabotropic glutamate receptors mGluR1a and mGluR5) concentrated in the postsynaptic membrane. The timely removal of glutamate from the synaptic cleft is critical to preventing desensitization resulting from continued exposure of the postsynaptic receptors to glutamate. Removal of glutamate from the synaptic cleft is mediated by a class of molecules known as glutamate transporter proteins located on surrounding astroglia and neurons. Five distinct, high-affinity, sodium-dependent glutamate transporters have been identified in animal and human central nervous system. Rat GLAST,

GLT-1, EAAC1 (EAAT1, EAAT2 and EATT3, respectively, in human), EAAT4 and EAAT5 differ in structure, pharmacological properties and tissue distribution.

Glutamate transport is a sodium- and potassium-coupled process capable of concentrating intracellular glutamate up to 10,000-fold compared with the extracellular environment. The stoichiometry of the process has been studied and at several models exist proposing various ionic exchanges. In one model derived from salamander retinal glial cells, the transport process is coupled to the co-transport of two sodium ions and the counter-transport of one potassium ion and one hydroxyl ion. (Bouvier et al. (1992), Nature 360:471-474). Another model proposes that with EAAC1, one glutamate is co-transported with three sodium ions and one hydrogen ion, with the counter-transport of one potassium ion (Zerangue *et al,* Nautre (1996) 383:634-637). Yet another study suggests that two sodium ions are co-transported with one glutamate molecule (Hart *et al.,* Science (1998) 280:2112-2114).

The cloning of glutamate transporter subtypes and detailed electrophysiological studies of these proteins reveals that glutamate transporters also possess channel-like properties. The conduct chloride flux is not thermodynamically coupled to substrate transport, although at transportable substrate is required for the chloride conductance. The binding of glutamate to the transporter may change its conformational state to form the chloride channel.

In addition to their possible role in development and learning (due to their potential for modulating normal synaptic transmission), the regulation of synaptic glutamate transporters is likely to play an important role in acute and chronic neurological processes. They can be involved through the disruption of synaptic transmission as well as through glutamate mediated excitotoxicity. Several diseases are associated with disruptions in glutamate transport.

Loss of cerebellar Purkinje cell is the hall mark of several inherited neurodegenerative diseases, including the trinucleotide repeat diseases such as spinocerebellar ataxia type 1 (SCA1), and is commonly associated with neurotoxicity of chronic ethanol ingestion, and with certain paraneoplastic neurological disorders. Although the molecular event that initiates the disease is known-- a trinucleotide repeat -- the cellular mechanisms responsible for Purkinje cell degeneration is not

known. The selective loss of glutamate transporters such as EAAT4 could make the protein an attractive candidate for a downstream event.

Similarly, dysregulation of glutamate transporter EAAC1 could also have pathological consequences. EAAC1 has the unusual localization to GABA pre-synaptic terminals. This transport could serve as a precursor transporter, supplying extracellular glutamate for GABA re-synthesis. GABA normally is synthesized, via glutamate amino decarboxylase, from glutamate. The source of this glutamate has been traditionally thought to be cellular glutamate. However, the unique localization of the glutamate transporter to GABA terminals suggests that these transporters supply precurser glutamate for GABA re-synthesis. Thus, EAAC1 could serve as an important step in GABAergic neurotransmission. Modulation of GABAergic metabolism is associated with a number of neurological disorders, including epilepsy, tremors, and spasticity. In addition, some theories of schizophrenia include disturbances of glutamate and GABA metabolism.

Accordingly, there is a need in the art for compounds that regulate glutamate transport and in particular, compounds and molecules that interact with glutamate transporter proteins.

SUMMARY OF THE INVENTION

The present invention provides a family of proteins that interact with glutamate transporter proteins. Through their interaction with glutamate transporter proteins, Glutamate Transporter Associated Proteins modulate glutamate transport, and also effect cytoskeletal organization and stability as well as chloride flux.

In one embodiment of the invention, there is provided a substantially pure polypeptide characterized as modulating intracellular glutamate transport, interacting with a glutamate transporter protein, and having an expression pattern in the brain. In addition, the polypeptide can have at least one PDZ domain, at least one regulatory G-protein domain, at lest one pleckstrin homology domain, at least one proline-rich domain and at least one guanine exchange factor domain. The polypeptide can have at least one pleckstrin homology domain, at least one spectrin repeat and at least one α-actinin domain.

In an additional embodiment of the invention, there is provided a substantially pure polypeptide characterized as modulating intracellular glutamate transport; interacting with a glutamate transporter protein; having an expression pattern in neural non-neuronal tissues; having at least one kinase C domains; having four transmembrane domains; and being hydrophobic.

In another embodiment of the invention, there is provided a substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or conservative variants thereof.

In still another embodiment of the invention, there is provided an isolated polynucleotide selected from the group consisting of: (a) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:2; (b) a polynucleotide of (a), wherein T can be U; (c) a polynucleotide complementary to (a) or (b); (d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1; (e) degenerate variants of (a), (b), (c) or (d); and (f) a fragment of (a), (b), (c), (d) or (e) having at least 15 base pairs and hybridizes to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:2.

In yet another embodiment of the invention, there is provided an isolated polynucleotide selected from the group consisting of: (a) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:4; (b) a polynucleotide of (a), wherein T can be U; (c) a polynucleotide complementary to (a) or (b); (d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:3; (e) degenerate variants of (a), (b), (c) or (d); and (e) a fragment of (a), (b), (c), (d) or (e) having at least 15 base pairs and hybridizes to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:4.

In still another embodiment of the invention, there is provided an isolated polynucleotide selected from the group consisting of: (a) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:6; (b) a polynucleotide of (a), wherein T can be U; (c) a polynucleotide complementary to (a) or (b); (d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:5; (e) degenerate variants of (a), (b), (c) or (d); and (f) a fragment of (a), (b), (c),

(d) or (e) having at least 15 base pairs and hybridizes to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:6.

In still a further embodiment of the invention, there is provided an antibody that binds to a Glutamate Transporter Associated Protein or binds to immunoreactive fragments thereof. The antibody can be polyclonal or monoclonal.

In yet another embodiment of the invention, there is provided an expression vector comprising a polynucleotide encoding Glutamate Transporter Associated Protein, *e.g.,* SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or complementary nucleotides thereof and fragments thereof. The vectors can be virus derived or plasmid derived.

In another embodiment of the invention, there is provided a method for producing a Glutamate Transporter Associated Protein polypeptide by culturing a host cell containing a nucleotide encoding a Glutamate Transporter Associated Protein under conditions suitable for the expression of the polypeptide and recovering the polypeptide from the host cell culture.

In another embodiment of the invention, there is provided a substantially pure polypeptide that interacts with the amino acid sequence QEAELTLP (SEQ ID NO:9) or amino acid sequence GRGGNESVM (SEQ ID NO:10).

In still another embodiment of the invention, there is provided a substantially pure polypeptide that interacts with the amino acid sequence set forth in SEQ ID NO:12.

In still another embodiment of the invention, there is provided a substantially pure polypeptide that interacts with the amino acid sequence set forth in SEQ ID NO:13.

In an addition embodiment of the invention, there is provided a method for identifying a compound that modulates a cellular response mediated by a Glutamate Transporter Associated Protein. The method includes incubating the compound with a cell expressing a Glutamate Transporter Associated Protein and a glutamate transporter protein under conditions sufficient to permit the components to interact

and comparing a cellular response in the cell incubated with the compound with the cellular response of a cell not incubated with the compound.

In yet another embodiment of the invention, there is provided a method for identifying a compound that inhibits an interaction between a Glutamate Transporter Associated Protein and a glutamate transporter protein. The method includes contacting a Glutamate Transporter Associated Protein with a glutamate transporter protein in the presence of the compound and comparing the formation of a Glutamate Transporter Associated Protein-glutamate transporter protein complex in the presence of the compound with a formation of the complex in the absence of the compound.

In still another embodiment of the invention, there is provided a transgenic non-human animal having a transgene that expresses a Glutamate Transporter Associated Protein chromosomally integrated into the germ cells of the animal. An embodiment of the invention provides a method for producing such transgenic animals.

In another embodiment of the invention, there is provided a computer readable medium having stored thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and sequences substantially identical thereto.

In another embodiment of the invention, there is provided a computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and sequences substantially identical thereto.

In yet another embodiment of the invention, there is provided a method for comparing a first sequence to a reference sequence wherein said first sequence is a nucleic acid sequence selected from the group consisting SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID

NO:6 and sequences substantially identical thereto. The method comprises reading the first sequence and the reference sequence through use of a computer program which compares sequences, and determining differences between the first sequence and the reference sequence with the computer program.

In yet another embodiment of the invention there is provided a method for identifying a feature in a sequence wherein the sequence is selected from the group consisting of a nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 sequences substantially identical thereto, or a polypeptide sequence SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and sequences substantially identical thereto. The method includes reading the sequence through the use of a computer program which identifies features in sequences and identifying features in the sequences with the computer program.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show schematic representations of GTRAP4-41 and GTRAP4-48, respectively.

Figure 2 shows overlapping deletion mutants of the carboxy terminus of EAAT4 used to identify domains interacting with GTRAP4-41 and GTRAP4-48.

Figure 3A shows the effect of GTRAP4-41 and GTRAP4-48 on sodium-dependent glutamate uptake in transfected HEK-rEAAT4 cells. **Figure 3B** shows kinetic data which demonstrates that GTRAP4-41, in the presence of EAAT4, increases the V_{max} of glutamate uptake.

Figure 4A shows the effect of GTRAP3-18 on sodium-dependent glutamate transport in transfected HEK-293 cells. **Figure 4B** shows that the effect of GTRAP 3-18 on EAAC1-mediated glutamate transport is specific.

Figures 5A and 5B show the effect of Glutamate Transporter Associated Proteins (GTRAPs) on glutamate transporter protein expression.

Figures 6A-C show the effect of GTRAPs on glutamate transporter protein activity.

Figures 7A and 7B show the interaction between GTRAP4-48 and RhoGEF.

Figures 8A-C show the effects of GTRAP3-18 antisense oligonucleotide on glutamate transport.

Figures 9A-E show the effect of retinoic acid on GTRAP3-18-mediated glutamate transport.

Figure 10 is a flow diagram illustrating a computer system, data retrieving device and display.

Figure 11 is a flow diagram illustrating one embodiment of process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database.

Figure 12 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous.

Figure 13 is a flow diagram illustrating one embodiment of a process 300 for comparing features in polynucleotide and polypeptide sequences.

Figure 14 (A-C) shows a nucleic acid sequence of a polynucleotide encoding GTRAP4-41.

Figure 15 shows an amino acid sequence of GTRAP4-41.

Figure 16 (A and B) shows a nucleic acid sequence of a polynucleotide encoding GTRAP4-48.

Figure 17 shows an amino acid sequence of GTRAP4-48.

Figure 18 shows a nucleic acid sequence of a polynucleotide encoding GTRAP3-18.

Figure 19 shows an amino acid sequence of GTRAP3-18.

Figure 20 (A and B) shows a nucleic acid sequence of a polynucleotide encoding PCTAIRE-1.

Figure 21 shows an amino acid sequence of PCTAIRE-1a.

Figure 22 shows an amino acid sequence of PCTAIRE1b.

DETAILED DESCRIPTION OF THE INVENTION

The identification of molecules regulating the transport of neurotransmitters is central to understanding the mechanisms of neural activity, synaptic plasticity and learning. Efficient and rapid removal of neurotransmitters from the synaptic cleft by neurotransmitter transporters is critical to synaptic transmission. Re-uptake of glutamate by glutamate transporters both terminates the synaptic action of glutamate, thereby preventing glutamate-mediated exotoxicity and recaptures glutamate molecules for possible reuse.

Accordingly, one embodiment of the invention provides a substantially pure polypeptide characterized as modulating intracellular glutamate transport, interacting with a glutamate transporter protein and having an expression pattern in the brain. A polypeptide molecule having such characteristics is known as a Glutamate Transporter Associated Protein (GTRAP). Glutamate Transporter Associated Proteins can be further characterized as having at least one PDZ domain, having at least one regulatory G-protein domain, having at least one pleckstrin homology domain, having at least one proline-rich domain, and having at least one guanine exchange factor domain. Glutamate Transporter Associated Protein can also be characterized as having at least one pleckstrin homology domain, having at least one spectrin repeat, and having at least one α-actinin domain.

Glutamate Transporter Associated Proteins modulate glutamate transport. Glutamate transport refers to the active movement of glutamate across a cellular membrane. Glutamate transport is an essential component of central nervous system glutamatergic neurotransmission. For example, glutamate transport is essential in the inactivation of synaptically released glutamate and the prevention of excitotoxicity. The concentration of glutamate is higher in the terminal than in the synaptic cleft, even following neurotransmitter release. Nonetheless, the transporters take up glutamate from the synaptic cleft and transport it into the cell. Glutamate transporters also serve to bring glutamate into the cell for use in cellular metabolism, e.g. provide glutamate for new synthesis of neurotransmitter GABA. GTRAPs associated with some types of glutamate transporter protein, for example, glutamate transporter protein EAAT4, stimulate glutamate transport. GTRAPs associated with other types of glutamate transporter proteins, for example, EAAC1, inhibit glutamate transport.

While not wishing to be bound to any one mechanism, the modulation in transport appears to be effected through a change in Vmax or a change in Km (see Examples section). Glutamate transporter proteins can signal messages to the cell about transport activities e.g. GTRAP48 activate G-protein signaling].

Glutamate Transporter Associated Proteins share several common features. All GTRAPs are able to interact with at least one glutamate transporter protein. Glutamate transporter proteins include GLAST, GLT-1, EAAC1, EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5. Glutamate transporters share over 50% amino acid sequence identity with each other, and display almost identical hydrophobic profiles including six prominent hydrophobic peaks, followed by a small hydrophobic peak and long hydrophobic stretch. The proteins are generally 500 to 600 amino acids in length, with high conservation of sequence in the transmembrane domain. The carboxyl and amino terminal domains are intracellular and have the least sequence conservation among all transporters. Less is known about the genomic structure of the transporter proteins. The glutamate transporter family is quite distinct in structure from the 12 transmembrane α-helix arrangement of another sodium- and chloride-dependent transporter family related to dopamine and serotonin transport. The glutamate transporter family transports L-glutamate, D-aspartate and L-aspartate and some other acidic amino acids such as threo-β-hydroxyaspartate (THA) and cysteate. However, the transporters display distinct properties in substrate or inhibitor selectivity, e.g. dihydrokainate is a specific inhibitor of GLT-1 and EAAC1 transports cysteine with much higher affinity than the other transporters. Various studies have suggested that transporters may form homomultimers, perhaps dimers, but physiological transport may only require monomers of the protein.

Immunohistochemical studies show that GLAST and GLT-1 (EAAT1 and EAAT2) are localized primarily in astrocytes. In the adult CNS, GLT-1 is widely distributed throughout the brain and spinal cord in astroglial cell bodies and processes, while GLAST protein is localized in glial cells of cerebellar molecular and granule cell layers, and in some astroglia throughout the brain . Double labeling post-embedding electron microscopic immunocytochemistry shows the two glial transporters, GLT-1 and GLAST, expressed in the same cell membrane. Each protein forms oligomeric complexes but GLT-1 and GLAST may not complex with each

other. Antisense knock-down studies show that these two glial transporters are responsible for over 80% of glutamate uptake in the brain, an observation later confirmed in GLT-1 null mice. Quantitative immunoblotting and electron microscopy indicate that the glial transporters are quite abundant; GLAST and GLT-1 respectively, are 2300 and 8500 molecules per μm^2 in CA1 hippocampus membrane, and 4700 and 740 molecules per μm^2 in the cerebellar molecular layer.

Developmental studies reveal differential expression of GLT-1 and GLAST mRNA and protein. Initially expression of GLAST predominates throughout the CNS, followed by a shift in expression to the cerebellum, whereas GLT-1 expression remains throughout most of the CNS. A dramatic up-regulation of GLT-1 gene expression at post-natal day 14 coincides with the post-natal development of glutamatergic transmission in the cortex.

GLT-1 mRNA and protein can, under certain conditions be found in neurons, *e.g.* cultured hippocampal neurons. Transiently localized GLT-1 on growing axons and axon pathways can also be detected. Additional studies in models of ischemic brain injury and in fetal ovine brain suggest rare neuronal expression of GLT-1 as well.

EAAC1 and EAAT4 are neuronal transporters. EAAC1 immunoreactivity is particularly high in regions such as the hippocampus, cerebellum and basal ganglia. It is widely distributed in neurons such as large cortical pyramidal neurons, and is also present in non-glutamatergic neurons including GABAergic cerebellar Purkinje cells. Ultra-structural studies suggest that EAAC1 is not a presynaptic transporter of glutamatergic neurons. In fact, EAAC1 appears to be primarily localized in the somatodendritic compartment, and is already expressed at stages preceding synaptic contact formation. Rarely, EAAC1 is found in pre-synaptic terminals, which are always inhibitory (e.g GABAergic). Ultra structurally, EAAC1 is present in dendrites and somas. Detailed EM -gold studies of synapses indicate that the protein is most often peri-synaptic in location, like EAAT4. EAAC1 is also widely expressed outside the central nervous system, so it may serve metabolic functions in neurons. For example, it may provide glutamate for resynthesis of GABA in GABAergic terminals, where the protein has been localized (Rothstein, *et al.* (1994) Neuron 13:713-725, herein incorporated by reference in its entirety). In fact, studies using

antisense oligonucleotides to inhibit EAAC1 suggest that this transporter may, in part, regulate GABA synthesis.

EAAT4 is largely expressed in the cerebellum with very faint levels of expression in hippocampus, neocortex, striatum, brain stem and thalamus, in both the adult human and rat CNS . EAAT4 is present at low concentrations in the synaptic membrane, but is highly enriched in the parts of the dendritic and spine membranes facing astrocytes. A functional relationship may exist between EAAT4 and the glial transporters, and that EAAT4, having a prominent Cl^--channel property, may function as a combined transporter and inhibitory glutamate receptor. The average density of EAAT4 protein in the Purkinje cell membrane has been calculated to be 1800 molecules per μ^2. Immunohistochemical as well as immunoblot analysis demonstrates that during development EAAT4 protein is expressed in the human cerebellum both pre- and post-natally, while its expression in the frontal cortex is restricted to fetal stages. In the cerebellum, Purkinje cells show faint EAAT4 immunoreactivity at gestation week 17. However, EAAT4 expression becomes increasingly intense from gestation week 23 to the infantile period. After the late infantile period, EAAT4 immunoreactivity shows the same pattern as in adults. The intracellular localization of EAAT4 also changes with development. In the early embryonic period, EAAT immunoreactivity is found in the short processes of the Purkinje cells, while in the late fetal to early infantile periods, EAAT4 immunoreactivity is found in the cell bodies and dendrites, and in the late infantile period, it is found in the spines.

Glutamate transporters and glutamate receptors are compartmentalized in and around the synaptic cleft and proteins capable of glutamate receptor membrane targeting and the epitopes responsible for these events are known. For example, three cytoplasmic molecules have been recently identified which bind to the final eight amino acids in the C-terminus of GluR2 and GluR3, but not to GluR1 or NR1. These molecules, named GRIP and ABP are all synaptically localized in the hippocampus and contain one or more PDZ domains, protein binding motifs of between 70 and 90 amino acids which have recently been implicated in the localization of other highly regulated proteins. None of these molecules interacts or regulates glutamate transporters.

Several studies document a role for neurons in modulating the expression and activity of glutamate transporters. Pathway lesion studies suggest that neurons can influence the astroglial (but not neuronal) expression of glutamate transporter subtypes. This has been validated in vitro, where astroglial (EAAT2) expression in cultured astrocytes appears to depend on neurons, most likely secreted factors, including glutamate itself. In fact, a number of trophic factors that modulate EAAT2 expression in various in vitro preparations have been identified. Protein kinase C phosphorylation of EAAT2 (GLT-1) has also been found to stimulate transport. Transporters can also be directly regulated through other signaling pathways. Activity of EAAC1 (and GABA and serotonin transporters) can be regulated through expression at the cell surface, via regulated cellular trafficking, occurring in part through protein kinase C and phosphatidylinositol 3-kinase pathways.

Glutamate Transporter Associated Proteins have an expression pattern in brain tissue. Immunofluorescence staining of brain tissue reveals a pattern of GTRAP immunoreactivity in brain tissue. Prominent immunolocalization is observed in the cerebellar cortex, especially in Purkinje cell somas and dendrites with no axonal localization. Expression is also observed in other brain regions including striatum, hippocampus and thalamus.

Expression of certain Glutamate Transporter Associated Proteins is observed outside the brain. For example, GTRAP3-18 is expressed in the liver, kidney, heart, muscle as well as in the central nervous system.

Glutamate Transporter Associated Proteins can include at least one PDZ domain, at least one regulatory G-protein domain, at least one pleckstrin homology domain (PH), at least one proline-rich domain, at least one guanine exchange factor domain (Dbl), at least one spectrin repeat and at least one α-actinin domain. Methods to identify such domains are known to those of skill in the art. For example, computer programs that compare invention nucleic acid and amino acid sequences to nucleic acid and amino acid sequences, and identify regions of homology can be used to identify such domains.

Exemplary Glutamate Transporter Associated Proteins of the invention include sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and

conservative variants thereof. The terms "conservative variation" and "substantially similar" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. The terms "conservative variation" and "substantially similar" also include the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

Also contemplated by the invention are polypeptides that share at least 90% sequence homology to the polypeptide sequences set forth as SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6. Sequence homology can be determined by those of skill in the art, for example, by computer programs that compare sequences such as Blast.

Exemplary polynucleotides encoding a Glutamate Transporter Associated Proteins are set forth as SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7. The term "polynucleotide", "nucleic acid", "nucleic acid sequence", or "nucleic acid molecule" refers to a polymeric form of nucleotides at least 10 bases in length. By "isolated polynucleotide" is meant a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA) independent of other sequences. The nucleotides of the invention can be deoxyribonucleotides, ribonucleotides in which uracil (U) is present in place of thymine (T), or modified forms of either nucleotide. The nucleotides of the invention can be complementary to the deoxynucleotides or to the ribonucleotides. A polynucleotide encoding a Glutamate Transporter Associated Protein includes "degenerate variants", sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one

codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of a polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1,SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 is functionally unchanged.

A nucleic acid molecule encoding a Glutamate Transporter Associated Protein includes sequences encoding functional Glutamate Transporter Associated Protein polypeptides as well as functional fragments thereof. As used herein, the term "functional polypeptide" refers to a polypeptide which possesses biological function or activity which is identified through a defined functional assay, and which is associated with a particular biologic, morphologic, or phenotypic alteration in the cell. The term "functional fragments of Glutamate Transporter Associated Protein," refers to fragments of a Glutamate Transporter Associated Protein that retain a Glutamate Transporter Associated Protein activity, *e.g.*, the ability to interact with a glutamate transporter protein, modulate intracellular glutamate transport, and the like. Additionally, functional Glutamate Transporter Associated Protein fragments may act as competitive inhibitors of Glutamate Transporter Associated Protein binding to a glutamate transporter protein, for example. Biologically functional fragments can vary in size from a polypeptide fragment as small as an epitope capable of binding an antibody molecule to a large polypeptide capable of participating in the characteristic induction or programming of phenotypic changes within a cell. Nucleotide fragments of the invention have at least 15 base pairs and hybridize to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:22.

Further embodiments of the invention provide isolated polynucleotides, wherein the nucleotide is at least 15 base pairs in length which hybridizes under moderately to highly stringent conditions to DNA encoding a polypeptide as set forth in SEQ ID NO:2 or to DNA encoding a polypeptide as set forth in SEQ ID NO:4, or SEQ ID NO:6. In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting

hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderately stringent conditions); and 0.1 x SSC at about 68°C (highly stringent conditions). Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

Antibodies of the invention may bind to Glutamate Transporter Associated Proteins provided by the invention to prevent normal interactions of Glutamate Transporter Associated Proteins. Binding of antibodies to Glutamate Transporter Associated Protein can interfere with for example, glutamate transport, with cytoskeletal stability by interfering with intracellular protein binding, with expression patterns of Glutamate Transporter Associated Proteins or with interactions with glutamate transporter proteins . Furthermore, binding of antibodies to Glutamate Transporter Associated Proteins can interfere with the localization of glutamate transporter proteins on cellular membranes.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA), the enzyme-linked immunosorbant assay (ELISA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will

know, or can readily discern, other immunoassay formats without undue experimentation.

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, F(ab')2, and Fv which are capable of binding to an epitopic determinant present in an invention polypeptide. Such antibody fragments retain some ability to selectively bind with its antigen or receptor.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988), incorporated herein by reference). Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, *Antibodies: A Laboratory Manual*, page 726 (Cold Spring Harbor Pub. 1988), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen/ligand, verifying the presence of antibody production by analyzing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. *See, e.g.*, Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, "Purification of Immunoglobulin G (IgG)" in *Methods In Molecular Biology*, VOL. 10, pages 79-104 (Humana Press 1992).

Antibodies which bind to an invention Glutamate Transporter Associated Protein polypeptide can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. For example, it may be desirable to produce antibodies that specifically bind to the amino- or carboxyl-terminal domains of an invention polypeptide. For the preparation of polyclonal antibodies, the polypeptide or peptide used to immunize an animal is derived from translated cDNA or chemically synthesized and can be conjugated to a carrier protein,

if desired. Commonly used carrier proteins which may be chemically coupled to the immunizing peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), tetanus toxoid, and the like.

Invention polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See, for example, Coligan, *et al.*, Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994, incorporated herein by reference).

The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptides of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention . Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

In using the monoclonal and polyclonal antibodies of the invention for the in vivo detection of antigen, *e.g.,* a Glutamate Transporter Associated Protein, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the antibodies are specific.

The concentration of detectably labeled antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled antibody for in vivo treatment or diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

A polynucleotide agent can be contained in a vector, which can facilitate manipulation of the polynucleotide, including introduction of the polynucleotide into a target cell. The vector can be a cloning vector, which is useful for maintaining the polynucleotide, or can be an expression vector, which contains, in addition to the polynucleotide, regulatory elements useful for expressing the polynucleotide and, where the polynucleotide encodes a peptide, for expressing the encoded peptide in a particular cell. An expression vector can contain the expression elements necessary to achieve, for example, sustained transcription of the encoding polynucleotide, or the regulatory elements can be operatively linked to the polynucleotide prior to its being cloned into the vector.

An expression vector (or the polynucleotide) generally contains or encodes a promoter sequence, which can provide constitutive or, if desired, inducible or tissue specific or developmental stage specific expression of the encoding polynucleotide, a poly-A recognition sequence, and a ribosome recognition site or internal ribosome entry site, or other regulatory elements such as an enhancer, which can be tissue

specific. The vector also can contain elements required for replication in a prokaryotic or eukaryotic host system or both, as desired. Such vectors, which include plasmid vectors and viral vectors such as bacteriophage, baculovirus, retrovirus, lentivirus, adenovirus, vaccinia virus, semliki forest virus and adeno-associated virus vectors, are well known and can be purchased from a commercial source (Promega, Madison WI; Stratagene, La Jolla CA; GIBCO/BRL, Gaithersburg MD) or can be constructed by one skilled in the art (see, for example, Meth. Enzymol., Vol. 185, Goeddel, ed. (Academic Press, Inc., 1990); Jolly, Canc. Gene Ther. 1:51-64, 1994; Flotte, J. Bioenerg. Biomemb. 25:37-42, 1993; Kirshenbaum et al., J. Clin. Invest. 92:381-387, 1993; each of which is incorporated herein by reference).

A polynucleotide useful in a method of the invention also can be operatively linked to tissue specific regulatory element, for example, a neuron specific regulatory element, such that expression of an encoded peptide agent is restricted to neurons in an individual, or to neurons in a mixed population of cells in culture, for example, an organ culture. For example, neuronal or glial promoters such as the myelin basic protein promoter, other neuronal-specific promoters, and astroglial promoters (e.g. GFAP- glial fibrillary acidic protein), known to those of skill in the art may be used. Muscle-regulatory elements including, for example, the muscle creatine kinase promoter (Sternberg et al., Mol. Cell. Biol. 8:2896-2909, 1988, which is incorporated herein by reference) and the myosin light chain enhancer/promoter (Donoghue et al., Proc. Natl. Acad. Sci., *USA* 88:5847-5851, 1991, which is incorporated herein by reference) are well known in the art. A variety of other promoters have been identified which are suitable for up regulating expression in cardiac tissue. Included, for example, are the cardiac I-myosin heavy chain (AMHC) promoter and the cardiac I-actin promoter. Other examples of tissue-specific regulatory elements include, tissue-specific promoters, pancreatic (insulin or elastase), and actin promoter in smooth muscle cells. Through the use of promoters, such as milk-specific promoters, recombinant retroviruses may be isolated directly from the biological fluid of the progeny.

A Glutamate Transporter Associated Protein polynucleotide of the invention can be inserted into a vector, which can be a cloning vector or a recombinant

expression vector. The term "expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of a polynucleotide, particularly, with respect to the present invention, a polynucleotide encoding all or a peptide portion of a Glutamate Transporter Associated Protein. Such expression vectors contain a promoter sequence, which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector generally contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to, the T7-based expression vector for expression in bacteria (Rosenberg, et al., Gene 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, J. Biol. Chem. 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter, which can be a T7 promoter, metallothionein I promoter, polyhedrin promoter, or other promoter as desired, particularly tissue specific promoters or inducible promoters.

Viral expression vectors can be particularly useful for introducing a polynucleotide useful in a method of the invention into a cell, particularly a cell in a subject. Viral vectors provide the advantage that they can infect host cells with relatively high efficiency and can infect specific cell types. For example, a polynucleotide encoding a Glutamate Transporter Associated Protein or functional peptide portion thereof can be cloned into a baculovirus vector, which then can be used to infect an insect host cell, thereby providing a means to produce large amounts of the encoded protein or peptide portion. The viral vector also can be derived from a virus that infects cells of an organism of interest, for example, vertebrate host cells such as mammalian, avian or piscine host cells. Viral vectors can be particularly useful for introducing a polynucleotide useful in performing a method of the invention into a target cell. Viral vectors have been developed for use in particular host systems, particularly mammalian systems and include, for example, retroviral vectors, other lentivirus vectors such as those based on the human immunodeficiency virus (HIV), adenovirus vectors, adeno-associated virus vectors, herpesvirus vectors, vaccinia virus vectors, and the like (see Miller and Rosman, BioTechniques 7:980-990, 1992; Anderson et al., Nature 392:25-30 Suppl., 1998; Verma and Somia, Nature

389:239-242, 1997; Wilson, New Engl. J. Med. 334:1185-1187 (1996), each of which is incorporated herein by reference).

When retroviruses, for example, are used for gene transfer, replication competent retroviruses theoretically can develop due to recombination of retroviral vector and viral gene sequences in the packaging cell line utilized to produce the retroviral vector. Packaging cell lines in which the production of replication competent virus by recombination has been reduced or eliminated can be used to minimize the likelihood that a replication competent retrovirus will be produced. All retroviral vector supernatants used to infect cells are screened for replication competent virus by standard assays such as PCR and reverse transcriptase assays. Retroviral vectors allow for integration of a heterologous gene into a host cell genome, which allows for the gene to be passed to daughter cells following cell division.

A polynucleotide, which can be contained in a vector, can be introduced into a cell by any of a variety of methods known in the art (Sambrook et al., Molecular Cloning: A laboratory manual (Cold Spring Harbor Laboratory Press 1989); Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1987, and supplements through 1995), each of which is incorporated herein by reference). Such methods include, for example, transfection, lipofection, microinjection, electroporation and, with viral vectors, infection; and can include the use of liposomes, microemulsions or the like, which can facilitate introduction of the polynucleotide into the cell and can protect the polynucleotide from degradation prior to its introduction into the cell. The selection of a particular method will depend, for example, on the cell into which the polynucleotide is to be introduced, as well as whether the cell is isolated in culture, or is in a tissue or organ in culture or *in situ*.

Introduction of a polynucleotide into a cell by infection with a viral vector is particularly advantageous in that it can efficiently introduce the nucleic acid molecule into a cell *ex vivo* or *in vivo* (see, for example, U.S. Patent No. 5,399,346, which is incorporated herein by reference). Moreover, viruses are very specialized and can be selected as vectors based on an ability to infect and propagate in one or a few specific cell types. Thus, their natural specificity can be used to target the nucleic acid molecule contained in the vector to specific cell types. As such, a vector based on an

HIV can be used to infect T cells, a vector based on an adenovirus can be used, for example, to infect respiratory epithelial cells, a vector based on a herpesvirus can be used to infect neuronal cells, and the like. Other vectors, such as adeno-associated viruses can have greater host cell range and, therefore, can be used to infect various cell types, although viral or non-viral vectors also can be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A polynucleotide sequence encoding a Glutamate Transporter Associated Protein can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing polynucleotides having eukaryotic or viral sequences in prokaryotes are well known in the art, as are biologically functional viral and plasmid DNA vectors capable of expression and replication in a host. Methods for constructing an expression vector containing a polynucleotide of the invention are well known, as are factors to be considered in selecting transcriptional or translational control signals, including, for example, whether the polynucleotide is to be expressed preferentially in a particular cell type or under particular conditions (see, for example, Sambrook et al., *supra*, 1989).

A variety of host cell/expression vector systems can be utilized to express a Glutamate Transporter Associated Protein coding sequence, including, but not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors; yeast cells transformed with recombinant yeast expression vectors; plant cell systems infected with recombinant virus expression vectors such as a cauliflower mosaic virus or tobacco mosaic virus, or transformed with recombinant plasmid expression vector such as a Ti plasmid; insect cells infected with recombinant virus expression vectors such as a baculovirus; animal cell systems infected with recombinant virus expression vectors such as a retrovirus, adenovirus or vaccinia virus vector; and transformed animal cell systems genetically engineered for stable expression. Where the expressed Glutamate Transporter Associated Protein is post-translationally modified, for example, by glycosylation, it can be particularly advantageous to select a host cell/expression vector system that can effect the desired modification, for example, a mammalian host cell/expression vector system.

Depending on the host cell/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, and the like can be used in the expression vector (Bitter et al., Meth. Enzymol. 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage Σ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like can be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells, for example, a human or mouse metallothionein promoter, or from mammalian viruses, for example, a retrovirus long terminal repeat, an adenovirus late promoter or a vaccinia virus 7.5K promoter, can be used. Promoters produced by recombinant DNA or synthetic techniques can also be used to provide for transcription of the inserted GDF receptors coding sequence.

In yeast cells, a number of vectors containing constitutive or inducible promoters can be used (see Ausubel et al., *supra*, 1987, see chapter 13; Grant et al., Meth. Enzymol. 153:516-544, 1987; Glover, DNA Cloning Vol. II (IRL Press, 1986), see chapter 3; Bitter, Meth. Enzymol. 152:673-684, 1987; see, also, The Molecular Biology of the Yeast *Saccharomyces* (Eds., Strathern et al., Cold Spring Harbor Laboratory Press, 1982), Vols. I and II). A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL can be used (Rothstein, DNA Cloning Vol. II (*supra*, 1986), chapter 3). Alternatively, vectors can be used which promote integration of foreign DNA sequences into the yeast chromosome.

Eukaryotic systems, particularly mammalian expression systems, allow for proper post-translational modifications of expressed mammalian proteins. Eukaryotic cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, phosphorylation, and advantageously, plasma membrane insertion of the gene product can be used as host cells for the expression of a Glutamate Transporter Associated Protein, or functional peptide portion thereof.

Mammalian cell systems which utilize recombinant viruses or viral elements to direct expression can be engineered. For example, when using adenovirus expression vectors, the Glutamate Transporter Associated Protein coding sequence can be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. Alternatively, the vaccinia virus 7.5K

promoter can be used (Mackett et al., Proc. Natl. Acad. Sci., *USA* 79:7415-7419, 1982; Mackett et al., J. Virol. 49:857-864, 1984; Panicali et al., Proc. Natl. Acad. Sci., *USA* 79:4927-4931, 1982). Particularly useful are bovine papilloma virus vectors, which can replicate as extrachromosomal elements (Sarver et al., Mol. Cell. Biol. 1:486, 1981). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host cell chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as, for example, the neo gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the Glutamate Transporter Associated Protein gene in host cells (Cone and Mulligan, Proc. Natl. Acad. Sci., *USA* 81:6349-6353, 1984). High level expression can also be achieved using inducible promoters, including, but not limited to, the metallothionein IIA promoter and heat shock promoters.

For long term, high yield production of recombinant proteins, stable expression is preferred. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with Glutamate Transporter Associated Protein cDNA controlled by appropriate expression control elements such as promoter, enhancer, sequences, transcription terminators, and polyadenylation sites, and a selectable marker. The selectable marker in the recombinant plasmid can confer resistance to the selection, and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which, in turn can be cloned and expanded into cell lines. For example, following the introduction of foreign DNA, engineered cells can be allowed to grow for 1 to 2 days in an enriched media, and then are switched to a selective media. A number of selection systems can be used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, Proc. Natl. Acad. Sci., *USA* 48:2026, 1982), and adenine phosphoribosyltransferase (Lowy, et al., Cell 22:817, 1980) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., Proc. Natl. Acad. Sci. *USA* 77:3567, 1980; O'Hare et al., Proc. Natl. Acad. Sci., *USA* 78: 1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and

Berg, Proc. Natl. Acad. Sci., *USA* 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., J. Mol. Biol. 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147, 1984) genes. Additional selectable genes, including trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman and Mulligan, Proc. Natl. Acad. Sci., *USA* 85:8047, 1988); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, Curr. Comm. Mol. Biol. (Cold Spring Harbor Laboratory Press, 1987), also have been described.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors can be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding Glutamate Transporter Associated Proteins of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (Gluzman, Eukaryotic Viral Vectors (Cold Spring Harbor Laboratory Press, 1982)).

The invention provides a method for producing a polypeptide characterized as interacting with a glutamate transporter protein; modulating intracellular glutamate transport; having an expression pattern in Purkinje cells of the brain; and being hydrophobic. The invention also provides a method for producing a polypeptide encoded by the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 or fragments thereof, including culturing the host cell under conditions suitable for the expression of the polypeptide and recovering the polypeptide from the host cell culture.

A Glutamate Transporter Associated Protein polypeptide or a fragment thereof, can be encoded by a recombinant or non-recombinant nucleic acid molecule and expressed in a cell. Preparation of a Glutamate Transporter Associated Protein polypeptide by recombinant methods provides several advantages. In particular, the

nucleic acid sequence encoding the Glutamate Transporter Associated Protein polypeptide can include additional nucleotide sequences encoding, for example, peptides useful for recovering the Glutamate Transporter Associated Protein polypeptide from the host cell. A Glutamate Transporter Associated Protein polypeptide can be recovered using well known methods, including, for example, precipitation, gel filtration, ion exchange, reverse-phase, or affinity chromatography (see, for example, Deutscher et al., "Guide to Protein Purification" in Meth. Enzymol., Vol. 182, (Academic Press, 1990)). Such methods also can be used to purify a fragment of a Glutamate Transporter Associated Protein polypeptide, for example, a particular binding sequence, from a cell in which it is naturally expressed.

A recombinant nucleic acid molecule encoding a Glutamate Transporter Associated Protein polypeptide or a fragment thereof can include, for example, a protease site, which can facilitate cleavage of the Glutamate Transporter Associated Protein polypeptide from a non-Glutamate Transporter Associated Protein polypeptide sequence, for example, a tag peptide, secretory peptide, or the like. As such, the recombinant nucleic acid molecule also can encode a tag peptide such as a polyhistidine sequence, a FLAG peptide (Hopp et al., Biotechnology 6:1204 (1988)), a glutathione S-transferase polypeptide or the like, which can be bound by divalent metal ions, a specific antibody (U.S. Patent No. 5,011,912), or glutathione, respectively, thus facilitating recovery and purification of the Glutamate Transporter Associated Protein polypeptide comprising the peptide tag. Such tag peptides also can facilitate identification of the Glutamate Transporter Associated Protein polypeptide through stages of synthesis, chemical or enzymatic modification, linkage, or the like. Methods for purifying polypeptides comprising such tags are well known in the art and the reagents for performing such methods are commercially available.

A nucleic acid molecule encoding a Glutamate Transporter Associated Protein polypeptide can be engineered to contain one or more restriction endonuclease recognition and cleavage sites, which can facilitate, for example, substitution of an element of the Glutamate Transporter Associated Protein polypeptide such as the selective recognition domain or, where present, a spacer element. As such, related Glutamate Transporter Associated Protein polypeptides can be prepared, each having a similar activity, but having specificity for different function-forming contexts. A

restriction endonuclease site also can be engineered into (or out of) the sequence coding a peptide portion of the Glutamate Transporter Associated Protein polypeptide, and can, but need not change one or more amino acids encoded by the particular sequence. Such a site can provide a simple means to identify the nucleic acid sequence, based on cleavage (or lack of cleavage) following contact with the relevant restriction endonuclease, and, where introduction of the site changes an amino acid, can further provide advantages based on the substitution.

In another embodiment of the invention there is provided a substantially pure polypeptide which interacts with amino acid sequence QEAELTLP (SEQ ID NO:9) or the amino acid sequence GRGGNESVM (SEQ ID NO:10). In a preferred embodiment, polypeptides interact with a Glutamate Transporter Associated Protein encoded by a polynucleotide that hybridizes to SEQ ID NO:1. An exemplary protein containing amino acid sequences QEAELTLP (SEQ ID NO:9) and GRGGNESVM (SEQ ID NO:10) is glutamate transport protein EAAT4 (see Examples). In another embodiment of the invention, there is provided a polynucleotide encoding a substantially pure polypeptide which interacts with amino acid sequence QEAELTLP (SEQ ID NO:9) or the amino acid sequence GRGGNESVM (SEQ ID NO:10).

Another embodiment of the invention provides a substantially pure polypeptide which interacts with a polypeptide having the sequence of amino acids found at amino acid residues 527 to 534 of EAAT4 (SEQ ID NO:9). Still another embodiment of the invention provides a substantially pure polypeptide which interacts with a polypeptide sequence having the sequence of amino acid found at amino acid residues 555 to 561 of EAAT4 (SEQ ID NO:10).

Still another embodiment of the invention provides a substantially pure polypeptide which interacts with the amino acid sequence set forth in SEQ ID NO:12. Also provided is a substantially pure polypeptide which interacts with the amino acid sequence set forth in SEQ ID NO:13. Such amino acid sequence are used as "bait" sequences in yeast two-hybrid screen (See Example 1). Polypeptides identified in such screens are interacting proteins. Interacting proteins can mediate or modulate the activities of intracellular proteins.

A method is provided for identifying a compound that modulates a cellular response mediated by a Glutamate Transporter Associated Protein . The method includes incubating the compound with a cell expressing a Glutamate Transporter Associated Protein and a glutamate transporter protein under conditions sufficient to permit the compound to interact with the cell. The effect of the compound on the cellular response is determined, either directly or indirectly, and a cellular response is then compared with a cellular response of a control cell. A suitable control includes, but is not limited to, a cellular response of a cell not contacted with the compound. The cell may be any cell of interest, including but not limited to neuronal cells, glial cells, cardiac cells, bronchial cells, uterine cells, testicular cells, liver cells, renal cells, intestinal cells, cells from the thymus and spleen, placental cells, endothelial cells, endocrine cells including thyroid, parathyroid, pituitary and the like, smooth muscle cells and skeletal muscle cells. The term "incubating" includes conditions which allow contact between the test compound and the cell of interest. "Contacting" may include in solution or in solid phase.

The cellular response can be an increase in glutamate transport or a decrease in glutamate transport. Glutamate transport can be assessed by measuring glutamate uptake assays (see Example 8) and other assays known in the art.

The cellular response can be an increase in cytoskeletal stability or a decrease in cytoskeletal stability. Cytoskeletal stability can be assessed for example, by examining the formation and maintenance of intracellular protein interaction, cell-surface receptor clustering, clustering of glutamate transporter proteins, and the like. Methods for demonstrating such cellular responses are well known in the art (*e.g.* biochemical methods and histological methods). (See Kornau *et al.* (1997) *Curr. Opin. Neurobiol.* 7:368-373; and Huganir *et al.* (2000) *Trends in Cell Biol.* 10:274-280, each of which are herein incorporated by reference in their entirety and Examples section for additional methodology).

The cellular response can be an increase in chloride flux or a decrease in chloride flux. Chloride flux can be assessed by methods known to those of skill in the art such as electrophysiological methods including, but not limited to, patch clamp analysis.

Glutamate Transporter Associated Proteins contemplated for use in the invention method includes, for example, GTRAP4-41, GTRAP4-48, PCTAIRE-1a, PCTAIRE-1b, and GTRAP3-18. Glutamate transport proteins contemplated for use in the invention method include GLAST, GLT-1, EAAC1, EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5.

In one preferred embodiment of the invention, the glutamate transport protein is EATT4 and the Glutamate Transporter Associated Protein is GTRAP4-41, GTRAP4-48, PCTAIRE-1a or PCTAIRE-1b. In another embodiment of the invention, the glutamate transport protein is EAAC1 and the Glutamate Transporter Associated Protein is GTRAP3-18.

In an embodiment of the invention, the cell expressing a Glutamate Transporter Associated Protein further expresses a RhoGEF protein. The Rho family of GTP-binding proteins regulates the rearrangement of the actin cytoskeleton. At least one Glutamate Transporter Associated Protein has a domain that permits interaction with a a guanine nucleotide exchange factor (GEF).

Compounds which modulate a cellular response include peptides, peptidomimetics, polypeptides, pharmaceuticals, chemical compounds and biological agents, for example. Antibodies, anti-epileptic compounds and combinatorial compound libraries can also be tested using the method of the invention. One class of organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups.

The test agent may also be a combinatorial library for screening a plurality of compounds. Compounds such as peptides identified in the method of the invention can be further cloned, sequenced, and the like, either in solution of after binding to a solid support, by any method usually applied to the isolation of a specific DNA

sequence Molecular techniques for DNA analysis (Landegren *et al.*, *Science* 242:229-237, 1988) and cloning have been reviewed (Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1998, herein incorporated by reference).

Candidate compounds are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc., to produce structural analogs. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

A variety of other agents may be included in the screening assay. These include agents like salts, neutral proteins, *e.g.*, albumin, detergents, *etc.* that are used to facilitate optimal protein-protein binding and/or reduce nonspecific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, antimicrobial agents and the like may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 10 h will be sufficient.

Another embodiment of the invention provides a method for identifying a compound that can inhibit an interaction between a Glutamate Transporter Associated Protein and a glutamate transporter protein. The method includes contacting a Glutamate Transporter Associated Protein with a glutamate transporter protein in the

presence of the compound, and comparing the formation of a Glutamate Transporter Associated Protein-glutamate transporter complex in the presence eof the compound with the formation of the complex in the absence of the compound. Compounds that affect complex formation include peptides, polypeptides, pepidomimetics, chemical compounds and biological agents.

Contacting includes in solution and solid phase. In a preferred embodiment, isolated Glutamate Associated Transporter Proteins are utilized. However, partially purified proteins, fractions of cell extracts, whole cell extracts, or intact cells may be utilized with the method of the invention.

The complex of the Glutamate Associated Transporter Protein and the glutamate transporter protein can be separated from uncomplexed components by conventional means, well known to one of skill in the art. Separation can be accomplished by size separation, physical separation, antibody-mediated separation, or other standard methods. For example, immunoprecipitation or gel electrophoresis can be used to separate Glutamate Transporter Associated Protein-glutamate transporter protein complex from components that are not part of the complex (See Examples section for details).

Also provided is a method of modulating glutamate transport in a subject in need thereof. The method includes administering to the subject a therapeutically effective amount of a compound that modulates expression or activity of a Glutamate Transporter Associated Protein, thereby modulating glutamate transport.

A method is further provided for treating a subject with a disorder associated with glutamate transport comprising administering to the subject a therapeutically effective amount of a compound that modulates Glutamate Transporter Associated Protein activity or interaction with glutamate transporter protein.

Essentially, any disorder that is etiologically linked to glutamate transport or to a Glutamate Transporter Associated Protein could be considered susceptible to treatment with an agent that modulates Glutamate Transporter Associated Protein activity. The disorder may be a neuronal cell disorder. Examples of neuronal cell disorders include but are not limited to epilepsy, neurodegenerative disease (e.g. Alzheimer's disease, Huntington's disease, Amyotrophic lateral sclerosis, Parkinson's disease), spinocerebellar ataxia (SCA), especially of the SCA type 1, multiple

sclerosis, disorders of neurotransmitter metabolism, including GABA metabolism and the like, Alzheimer's disease, Parkinson's disease, stroke, and brain or spinal cord injury/damage, including ischemic injury, and the like. Disorders also include glutamate toxicity, a disorder of memory, a disorder of learning or a disorder of brain development, and the like. Also included are disorders of glutamate-GABA imbalance such as schizophrenia, and the like.

In a preferred embodiment, the Glutamate Transporter Associated Protein is GTAP4-41, GTRAP4-48 or PCTAIRE-1 (including PCTAIRE-1a and PCTAIRE-1b) and the disorder is a disorder of the nervous system such as neurodegeneration or spinocerebellar ataxia type 1.

When the Glutamate Transporter Associated Protein is GTRAP3-18 the disorder is epilepsy or a disorder of GABA metabolism (e.g. tremors, spasticity, schizophrenia), for example.

Treatment can include modulation of Glutamate Transporter Associated Protein expression or activity by administration of a therapeutically effective amount of a compound that modulates Glutamate Transporter Associated Protein or Glutamate Transporter Associated Protein activity. The term "modulate" envisions the suppression of Glutamate Transporter Associated Protein activity or expression when the Glutamate Transporter Associated Protein is overexpressed or has an increased activity as compared to a control. The term "modulate" also includes the augmentation of the expression of Glutamate Transporter Associated Protein when it is underexpressed or has a decreased activity as compared to a control. The term "compound" as used herein describes any molecule, *e.g.*, protein, nucleic acid, or pharmaceutical, with the capability of altering the expression of Glutamate Transporter Associated Protein polynucleotide or activity of Glutamate Transporter Associated Protein. Treatment can inhibit the transcription or translation of a Glutamate Transporter Associated Protein nucleotide sequence, inhibit the interaction of a domain of Glutamate Transporter Associated Protein with its target protein, may increase the avidity of this interaction by means of allosteric effects, may block the binding activity of a domain of Glutamate Transporter Associated Protein or influence other functional properties of Glutamate Transporter Associated Proteins.

Candidate agents include nucleic acids that interfere with expression of Glutamate Transporter Associated Protein , such as an antisense nucleic acid, ribozymes, and the like. Candidate agents also encompass numerous chemical classes wherein the agent modulates Glutamate Transporter Associated Protein expression or activity. For example, when the Glutamate Transporter Associated Protein is GTRAP3-18, the compound can be a polynucleotide having a nucleic acid sequence substantially similar to SEQ ID NO:20 (5'-GAGCGGGGCAAGGTTCAC-3'). A nucleotide encoded by SEQ ID NO:20 is antisense to the nucleic acid sequence of GTRRAP3-18 (See Example 13). GTRAP3-18 can also be modulated by retinoic acid (See Example 14).

When the Glutamate Transporter Associated Protein is GTRAP4-41, GTRAP4-48 or PCTAIRE-1, modulatory compounds include a polynucleotide having a nucleic acid sequence that is substantially similar to an antisense nucleic acid sequence that binds to a polynucleotide encoding GTRAP4-41, GTRAP4-48 or PCTAIRE-1.

Modulation of glutamate transport can be an increase in glutamate transport or a decrease in glutamate transport. When a disorder is associated with an increase in glutamate transport, compounds that decrease glutamate transport can be used. For example, compounds that modulate expression of GTRAP3-18 are contemplated. When a disorder is associated with a decrease in glutamate transport, compound that increase glutamate transport are contemplated. For example, compounds that modulate expression of GTRAP4-41, GTRAP4-48, or PCTAIRE-1 (a and b) are contemplated.

Detection of altered (decreased or increased) levels of Glutamate Transporter Associated Protein expression can be accomplished by hybridization of nucleic acids isolated from a cell of interest with a Glutamate Transporter Associated Protein of the invention. Analysis, such as Northern Blot analysis, are utilized to quantitate expression of Glutamate Transporter Associated Protein , such as to measure Glutamate Transporter Associated Protein transcripts. Other standard nucleic acid detection techniques will be known to those of skill in the art. Detection of altered levels of Glutamate Transporter Associated Protein can also accomplished using assays designed to detect Glutamate Transporter Associated Protein polypeptide. For

example, antibodies or peptides that specifically bind a Glutamate Transporter Associated Protein polypeptide can be utilized. Analyses, such as radioimmune assay or immunohistochemistry, are then used to measure Glutamate Transporter Associated Protein , such as to measure protein concentration qualitatively or quantitatively.

Where a disorder is associated with the increased expression of Glutamate Transporter Associated Protein , nucleic acid sequences that interfere with the expression of Glutamate Transporter Associated Protein can be used. This approach also utilizes, for example, antisense nucleic acid, ribozymes, or triplex agents to block transcription or translation of Glutamate Transporter Associated Protein mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving it with a ribozyme in disorders associated with increased Glutamate Transporter Associated Protein. Alternatively, a dominant negative form of Glutamate Transporter Associated Protein polypeptide could be administered.

When Glutamate Transporter Associated Protein is overexpressed, candidate agents include antisense nucleic acid sequences. Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, 1990, *Scientific American*, 262:40). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, 1988, *Anal. Biochem.*, 172:289).

Use of an oligonucleotide to stall transcription is known as the triplex strategy since the oligomer winds around double-helical DNA, forming a three-strand helix. Therefore, these triplex compounds can be designed to recognize a unique site on a chosen gene (Maher, *et al.*, 1991, *Antisense Res. and Dev.*, 1(3):227; Helene, C., 1991, *Anticancer Drug Design*, 6:569).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988, *J. Amer. Med. Assn.*, 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, 1988, *Nature*, 334:585) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

When a disorder is associated with the decreased expression of Glutamate Transporter Associated Protein, nucleic acid sequences that encode Glutamate Transporter Associated Protein can be used. An agent which modulates Glutamate Transporter Associated Protein expression includes a polynucleotide encoding a polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:22, or a conservative variant thereof. Alternatively, an agent of use with the subject invention includes agents that increase the expression of a polynucleotide encoding Glutamate Transporter Associated Protein or an agent that increases the activity of Glutamate Transporter Associated Protein polypeptide.

In another series of embodiments, the present invention provides transgenic animal models diseases or disorders associated with mutations in the Glutamate Transporter Associated Protein genes. The animal may be essentially any amphibian, reptile, fish, mammal, and the like. Preferably, the transgenic animal is mammalian including rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates. In addition, invertebrate models, including nematodes and insects, may be used for certain applications. The animal models are produced by

standard transgenic methods including microinjection, transfection, or by other forms of transformation of embryonic stem cells, zygotes, gametes, and germ line cells with vectors including genomic or cDNA fragments, minigenes, homologous recombination vectors, viral insertion vectors and the like. Suitable vectors include vaccinia virus, adenovirus, adeno associated virus, retrovirus, liposome transport, neuraltropic viruses, Herpes simplex virus, and the like. The animal models may include transgenic sequences comprising or derived from Glutamate Transporter Associated Proteins including normal and mutant sequences, intronic, exonic and untranslated sequences, and sequences encoding subsets of Glutamate Transporter Associated Protein such as functional domains.

The major types of animal models provided include: (1) Animals in which a normal human Glutamate Transporter Associated Protein gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; in which a normal human Glutamate Transporter Associated Protein gene has been recombinantly substituted for one or both copies of the animal's homologous Glutamate Transporter Associated Protein gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Glutamate Transporter Associated Protein genes have been recombinantly "humanized" by the partial substitution of sequences encoding the human homologue by homologous recombination or gene targeting. (2) Animals in which a mutant human Glutamate Transporter Associated Protein gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; in which a mutant human Glutamate Transporter Associated Protein gene has been recombinantly substituted for one or both copies of the animal's homologous Glutamate Transporter Associated Protein gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Glutamate Transporter Associated Protein genes have been recombinantly "humanized" by the partial substitution of sequences encoding a mutant human homologue by homologous recombination or gene targeting. (3) Animals in which a mutant version of one of that animal's Glutamate Transporter Associated Protein genes has been recombinantly

introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and/or in which a mutant version of one of that animal's Glutamate Transporter Associated Protein genes has been recombinantly substituted for one or both copies of the animal's homologous Glutamate Transporter Associated Protein gene by homologous recombination or gene targeting. (4) "Knock-out" animals in which one or both copies of one of the animal's Glutamate Transporter Associated Protein genes have been partially or completely deleted by homologous recombination or gene targeting, or have been inactivated by the insertion or substitution by homologous recombination or gene targeting of exogenous sequences.

In a preferred embodiment of the invention, there is provided a transgenic non-human animal having a transgene that expresses a Glutamate Transporter Associated Protein-encoding polynucleotide chromosomally integrated into the germ cells of the animal. Animals are referred to as "transgenic" when such animal has had a heterologous DNA sequence, or one or more additional DNA sequences normally endogenous to the animal (collectively referred to herein as "transgenes") chromosomally integrated into the germ cells of the animal. The transgenic animal (including its progeny) will also have the transgene fortuitously integrated into the chromosomes of somatic cells.

Various methods to make the transgenic animals of the subject invention can be employed. Generally speaking, three such methods may be employed. In one such method, an embryo at the pronuclear stage (a "one cell embryo") is harvested from a female and the transgene is microinjected into the embryo, in which case the transgene will be chromosomally integrated into both the germ cells and somatic cells of the resulting mature animal. In another such method, embryonic stem cells are isolated and the transgene incorporated therein by electroporation, plasmid transfection or microinjection, followed by reintroduction of the stem cells into the embryo where they colonize and contribute to the germ line. Methods for microinjection of mammalian species is described in United States Patent No. 4,873,191. In yet another such method, embryonic cells are infected with a retrovirus containing the transgene whereby the germ cells of the embryo have the transgene chromosomally integrated therein. When the animals to be made transgenic

are avian, because avian fertilized ova generally go through cell division for the first twenty hours in the oviduct, microinjection into the pronucleus of the fertilized egg is problematic due to the inaccessibility of the pronucleus. Therefore, of the methods to make transgenic animals described generally above, retrovirus infection is preferred for avian species, for example as described in U.S. Patent No. 5,162,215. If microinjection is to be used with avian species, however, a recently published procedure by Love *et al.*, (Biotechnology, 12, Jan 1994) can be utilized whereby the embryo is obtained from a sacrificed hen approximately two and one-half h after the laying of the previous laid egg, the transgene is microinjected into the cytoplasm of the germinal disc and the embryo is cultured in a host shell until maturity. When the animals to be made transgenic are bovine or porcine, microinjection can be hampered by the opacity of the ova thereby making the nuclei difficult to identify by traditional differential interference-contrast microscopy. To overcome this problem, the ova can first be centrifuged to segregate the pronuclei for better visualization.

The non-human animals of the invention are murine typically (*e.g.*, mouse). The transgenic non-human animals of the invention are produced by introducing "transgenes" into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for microinjection. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al., Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene.

The term "transgenic" is used to describe an animal which includes exogenous genetic material within all of its cells. A "transgenic" animal can be produced by cross-breeding two chimeric animals which include exogenous genetic material within cells used in reproduction. Twenty-five percent of the resulting offspring will be transgenic *i.e.*, animals which include the exogenous genetic material within all of their cells in both alleles. Fifty percent of the resulting animals will include the

exogenous genetic material within one allele and twenty five percent will include no exogenous genetic material.

In the microinjection method useful in the practice of the subject invention, the transgene is digested and purified free from any vector DNA *e.g.* by gel electrophoresis. It is preferred that the transgene include an operatively associated promoter which interacts with cellular proteins involved in transcription, ultimately resulting in constitutive expression. Promoters useful in this regard include those from cytomegalovirus (CMV), Moloney leukemia virus (MLV), and herpes virus, as well as those from the genes encoding metallothionine, skeletal actin, P-enolpyruvate carboxylase (PEPCK), phosphoglycerate (PGK), DHFR, and thymidine kinase. Promoters for viral long terminal repeats (LTRs) such as Rous Sarcoma Virus can also be employed. Constructs useful in plasmid transfection of embryonic stem cells will employ additional regulatory elements well known in the art such as enhancer elements to stimulate transcription, splice acceptors, termination and polyadenylation signals, and ribosome binding sites to permit translation.

Retroviral infection can also be used to introduce transgene into a non-human animal, as described above. The developing non-human embryo can be cultured in vitro to the blastocyst stage. During this time, the blastomeres can be targets for retro viral infection (Jaenich, R., Proc. Natl. Acad. Sci USA 73:1260-1264, 1976). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.* (1986) in Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retro virus carrying the transgene (Jahner, *et al., Proc. Natl. Acad. Sci. USA* 82:6927-6931, 1985; Van der Putten, *et al., Proc. Natl. Acad. Sci USA* 82:6148-6152, 1985). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart, *et al., EMBO J.* 6:383-388, 1987). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoel (D. Jahner *et al., Nature* 2-98:623-628, 1982). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic nonhuman animal. Further, the founder may contain various retro viral insertions of

the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (D. Jahner *et al., supra*).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured in vitro and fused with embryos (M. J. Evans *et al. Nature* 292:154-156, 1981; M.O. Bradley *et al., Nature* 309: 255-258, 1984; Gossler, *et al., Proc. Natl. Acad. Sci USA* 83: 9065-9069, 1986; and Robertson *et al., Nature* 322:445-448, 1986). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retro virus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a nonhuman animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. (For review see Jaenisch, R., *Science* 240: 1468-1474, 1988).

"Transformed" means a cell into which (or into an ancestor of which) has been introduced, by means of recombinant nucleic acid techniques, a heterologous nucleic acid molecule. "Heterologous" refers to a nucleic acid sequence that either originates from another species or is modified from either its original form or the form primarily expressed in the cell.

"Transgene" means any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism (*i.e.*, either stably integrated or as a stable extrachromosomal element) which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism. Included within this definition is a transgene created by the providing of an RNA sequence which is transcribed into DNA and then incorporated into the genome. The transgenes of the invention include DNA sequences which encode Glutamate Transporter Associated Protein polypeptide-sense and antisense polynucleotides, which may be expressed in a transgenic non-human animal. The term "transgenic" as used herein additionally includes any organism whose genome has been altered by in vitro manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. As used herein, the

term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or "knocked out".

Another embodiment of the invention provides a computer readable medium having store thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:22 and sequences substantially identical thereto.

A further embodiment of the invention provides a computer system comprising a processor and a data storage device wherein said date storage device has stored thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:22 and sequences substantially identical thereto. The computer system, additionally can contain a sequence comparison algorithm and a data storage device having at least one reference sequence stored on it. The sequence comparison algorithm comprises a computer program which indicates polymorphisms. The term "polymorphism", as used herein, refers to the existence of multiple alleles at a single locus. Polymorphism can be are several types including, for example, those that change DNA sequence but do not change protein sequence, those that change protein sequence without changing function, those that create proteins with a different activity, and those that create proteins that are non-functional.

Embodiments of the invention include systems (*e.g.*, internet based systems), particularly computer systems which store and manipulate the coordinate and sequence information described herein. One example of a computer system 100 is illustrated in block diagram form in Figure 10. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze the coordinates and sequences as set forth herein. The computer system 100 typically includes a processor for processing, accessing and manipulating the sequence data. The processor 105 can be any well-known type of central processing unit, such as,

for example, the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq, AMD or International Business Machines.

Typically the computer system 100 is a general purpose system that comprises the processor 105 and one or more internal data storage components 110 for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

In one particular embodiment, the computer system 100 includes a processor 105 connected to a bus which is connected to a main memory 115 (preferably implemented as RAM) and one or more internal data storage devices 110, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system 100 further includes one or more data retrieving device 118 for reading the data stored on the internal data storage devices 110.

The data retrieving device 118 may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, or a modem capable of connection to a remote data storage system (*e.g.*, via the internet) etc. In some embodiments, the internal data storage device 110 is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system 100 may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

The computer system 100 includes a display 120 which is used to display output to a computer user. It should also be noted that the computer system 100 can be linked to other computer systems 125a-c in a network or wide area network to provide centralized access to the computer system 100.

Figure 11 is a flow diagram illustrating one embodiment of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system 100, or a public database such as GENBANK that is available through the Internet.

The process 200 begins at a start state 201 and then moves to a state 202 wherein the new sequence to be compared is stored to a memory in a computer system 100. As discussed above, the memory could be any type of memory, including RAM or an internal storage device.

The process 200 then moves to a state 204 wherein a database of sequences is opened for analysis and comparison. The process 200 then moves to a state 206 wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state 210 to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system.

Once a comparison of the two sequences has been performed at the state 210, a determination is made at a decision state 210 whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process 200.

If a determination is made that the two sequences are the same, the process 200 moves to a state 214 wherein the name of the sequence from the database is displayed to the user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process 200 moves to a decision state 218 wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process 200 terminates at an end state 220. However, if more sequences do exist in the database, then the process 200 moves to a state 224 wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database.

It should be noted that if a determination had been made at the decision state 212 that the sequences were not homologous, then the process 200 would move immediately to the decision state 218 in order to determine if any other sequences were available in the database for comparison.

Figure 12 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous. The process 250 begins at a start state 252 and then moves to a state 254 wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state 256. The process 250 then moves to a state 260 wherein the first character in the first sequence is read and then to a state 262 wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it is preferably in the single letter amino acid code so that the first and sequence sequences can be easily compared.

A determination is then made at a decision state 264 whether the two characters are the same. If they are the same, then the process 250 moves to a state 268 wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If they are, then the process 250 continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process 250 moves to a decision state 274 to determine whether there are any more characters either sequence to read.

If there are not any more characters to read, then the process 250 moves to a state 276 wherein the level of homology between the first and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%.

Homology or identity is often measured using sequence analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of

Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various deletions, substitutions and other modifications. The terms "homology" and "identity" in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same when compared and aligned for maximum correspondence over a comparison window or designated region as measured using any number of sequence comparison algorithms or by manual alignment and visual inspection.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequence for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443, 1970, by the search for similarity method of person & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection. Other algorithms for determining homology or identity include, for example, in addition to a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS

(Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BLocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic Algorithm) and WHAT-IF. Such alignment programs can also be used to screen genome databases to identify polynucleotide sequences having substantially identical sequences. A number of genome databases are available, for example, a substantial portion of the human genome is available as part of the Human Genome Sequencing Project (J. Roach, http://weber.u.Washington.edu/~roach/human_ genome_ progress 2.html) (Gibbs, 1995). At least twenty-one other genomes have already been sequenced, including, for example, *M. genitalium* (Fraser *et al.*, 1995), M. jannaschii (Bult *et al.*, 1996), *H. influenzae* (Fleischmann *et al.*, 1995), *E. coli* (Blattner *et al.*, 1997), and yeast (*S. cerevisiae*) (Mewes *et al.*, 1997), and *D. melanogaster* (Adams *et al.*, 2000). Significant progress has also been made in sequencing the genomes of model organism, such as mouse, *C. elegans*, and *Arabadopsis sp.* Several databases containing genomic information annotated with some functional information are maintained by different organization, and are accessible via the internet, for example, http://wwwtigr.org/tdb; http://www.genetics.wisc.edu; http://genome-www.stanford.edu/~ball; http://hiv-web.lanl.gov; http://www.ncbi.nlm.nih.gov; http://www.ebi.ac.uk; http://Pasteur.fr/other/biology; and http://www.genome.wi.mit.edu.

One example of a useful algorithm is BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, Nuc. Acids Res. 25:3389-3402, 1977, and Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). This algorithm involves first identifying high scoring

sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectations (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915, 1989) alignments (B) of 50, expectation (E) of 10, M=5, N= -4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873, 1993). One measure of similarity provided by BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a references sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

In one embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") In particular, five specific BLAST programs are used to perform the following task:

(1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;

(2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

(3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

(4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

(5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (*i.e.*, aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet *et al.*, Science 256:1443-1445, 1992; Henikoff and Henikoff, Proteins 17:49-61, 1993). Less preferably, the PAM or PAM250 matrices may also be used (see, *e.g.*, Schwartz and Dayhoff, eds., 1978, *Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure*, Washington: National Biomedical Research Foundation). BLAST programs are accessible through the U.S. National Library of Medicine, *e.g.*, at www.ncbi.nlm.nih.gov.

The parameters used with the above algorithms may be adapted depending on the sequence length and degree of homology studied. In some embodiments, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user.

Figure 13 is a flow diagram illustrating one embodiment of an identifier process 300 for detecting the presence of a feature in a sequence. The process 300

begins at a start state 302 and then moves to a state 304 wherein a first sequence that is to be checked for features is stored to a memory 115 in the computer system 100. The process 300 then moves to a state 306 wherein a database of sequence features is opened. Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG". Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group (www.gcg.com). Alternatively, the features may be structural polypeptide motifs such as alpha helices, beta sheets, or functional polypeptide motifs such as enzymatic active sites, helix-turn-helix motifs or other motifs known to those skilled in the art.

The following examples are intended to illustrate, but not limit, the invention.

EXAMPLE 1

Identification of Proteins Interacting With Glutamate Transporter Proteins

Yeast Two-Hybrid with EAAT4 Yeast two-hybrid screens were performed using the HF7c' yeast strain harboring the reporter genes HIS 3 and β-galactosidase (β-gal) under the control of GAL4 activation. The final 77 amino acids of EAAT4 (carboxy-intracellular domain SEQ ID NO:12) were subcloned in-frame into pGBT9 (GAL4 binding domain vector, CLONTECH) and used to screen a rat brain cDNA library constructed in pGAD10 (GAL4 activation domain vector, CLONTECH). The plasmids were transformed into HF7c' yeast cells and positive clones selected on triple-minus plates (Leu-, Trp-, His-) and assayed for β-galactosidase activity. Positive clones were co-transformed with either the bait vector or the original pGAD10 vector into yeast cells to confirm the interaction. For a subsequent EAAT4 C-terminal domain analysis, different regions of the final 77 amino acids of EAAT4 were subcloned in-frame into the pGBT9 vector.

Yeast Two-Hybrid Screen with EAAC1 The MATCHMAKER Two-Hybrid System (Clontech) was used for screening. Using the carboxy-terminal intracellular domain of EAAC1 (the carboxy- 87 amino acids, cDNA position 1458-1719; SEQ ID NO:13) as bait in a yeast two-hybrid screen of an adult rat brain cDNA library, 78 clones displaying β-galactosidase activity were identified. Plasmid DNAs were

isolated from positive clones and re-co-transformed with bait cDNA back into yeast to reconfirm the interaction. Restriction and sequencing analyses revealed that ten of these clones with the strongest β-galactosidase activity were identical.

EXAMPLE 2

Isolation and Primary Structure of Glutamate Transporter Associated Proteins

Cloning of full-length GTRAP4-41 and GTRAP4-48 cDNAs. Marathon cDNA amplification (CLONTECH) was used to perform both 5′- and 3′-RACE on cDNA synthesized from rat brain poly(A)$^+$ RNA. The double-stranded cDNA was ligated to the Marathon cDNA Adaptor which contains an adaptor primer (AP1) binding site. The 1.1 kb GTRAP4-41and 1.4 kb GTRAP4-48 cDNA fragments identified using the yeast two-hybrid system were used to design gene-specific primers (GSPs) which could be used in 5′- and 3′-RACE PCR reactions along with the AP1 primer. The RACE products obtained were sequenced and new GSPs designed, generating a series of overlapping RACE products, which were joined together by PCR. Overlapping RACE products were put through ten cycles of denaturation, annealing and extension in the absence of primers. Nested primers were added and the PCR continued for a further 20 cycles to amplify the overlapped template.

GTRAP4-41 AND 4-48 Two independent cDNA clones were isolated and the proteins they encode were named GTRAP4-41 and GTRAP4-48 (for glutamate transporter 4 associated protein). Isolation of the full-length cDNAs by a series of 5' and 3' RACE PCR reactions demonstrated that the largest open reading frame (ORF) for GTRAP4-41 is 7,164 base pairs (SEQ ID NO:1), which encodes a 2,388 amino acid protein (SEQ ID NO:2) with a predicted relative molecular mass (M_r) of 270,958 Da (accession AF225960). A BLAST search of the GenBank database shows that GTRAP4-41 possesses 87% identity with β-spectrin III (accession AB008567). GTRAP4-41 possesses seventeen 16 amino acid spectrin repeats, two α-actinin domains and a pleckstrin homology domain (Fig. 1A).

The largest ORF identified for GTRAP4-48 (accession AF225961) is 4,581 base pairs (SEQ ID NO:3), which encodes a 1,527 amino acid protein (SEQ ID NO:4) with a predicted M_r of 168,698 Da. A BLAST search of the GenBank database shows

that GTRAP4-48 is unique, but it possesses significant homology to the KIAA0380 cDNA-encoded protein (90% identity) and the recently described RhoGEF, p115. GTRAP4-48 posses multiple potential interaction and regulatory domains (Figure 1B). GTRAP4-48 has a PDZ domain, a regulatory G-protein domain, a pleckstrin homology region (PH) and two proline-rich sequences (PRO). These regions have all been implicated in protein-protein interactions by interacting with the C termini of proteins and are thought to be important in the subcellular targeting of the interacting proteins (Katan *et al.,* FEBS Lett. (1999) 452:36-40; LeVine Mol.Neurobiol. (1999); 19:111-149). The function of PH domain is not clearly clarified, several putative functions have been suggested; (1) binding to the β/γ subunit of heterotrimeric G proteins, (2) binding to lipids, (3) binding to phosphorylated Ser/Thr residues, (4) attachment to membrane by an unknown mechanism. The protein multiple PKC phosphorylation sites and one tyrosine kinase phosphorylation site. It contains 3 helix- loop-helix signatures. Finally, the protein contains a separated tandem periodic repeat of SQPEGS of undermined significance.

GTRAP3-18. Following isolation, one clone, E18, was completely sequenced and the protein encoded is named GTRAP3-18 (glutamate transporter EAAC1 associated protein). Clone E18 is a full-length cDNA containing initiation methionine and polyA tail (SEQ ID NO:5). GTRAP3-18 encodes a protein of 188 amino acids (SEQ ID NO:6) with a calculated molecular mass of 22.5 kDa. Protein analysis indicates that it is a very hydrophobic protein with four possible transmembrane domains. Both the carboxy-terminal and amino-terminal domains contain protein kinase C motifs and may be intracellular. JWA protein (Genbank NP006398), a novel human differentially displayed vitamin A responsive gene, is 95% identical to GTRAP3-18, suggesting that GTRAP3-18 is a rat JWA protein homologue.

EXAMPLE 3

Antibodies

Generation of Polyclonal GTRAP4-41 and GTRAP4-48 Antibodies. Affinity purified polyclonal antisera to EAAT4, GTRAP4-41 and GTRAP4-48 were produced using methods identical to previous studies (Rothstein *et al.,* Neuron (1994) 13:713-725. Synthetic peptides corresponding to epitopes of EAAT4 (carboxy-terminal; EKGASRGRGGNESA; SEQ ID NO:14 and amino-terminal;

KNSLFLRESGAGGGCL; SEQ ID NO:15), rat GTRAP4-41 (KRGPAPSPMPQSRSSE; SEQ ID NO:16) and rat GTRAP4-48 (KTPERTSPSHHRQPSD; SEQ ID NO:17) were synthesized. Monospecific antibodies to GTRAP4-41 and 4-48 were produced.

The affinity-purified GTRAP4-41 antibodies recognize a 270 KDa protein in HEK 293T cells transfected with the full-length GTRAP4-41 cDNA and the affinity-purified GTRAP4-48 antibodies recognized a 170 KDa protein in HEK 293T cells transfected with the full-length GTRAP4-48 cDNA

Generation of Polyclonal GTRAP3-18 Antibodies. Affinity purified polyclonal antisera to GTRAP3-18 was produced as described in Rothstein *et al.* (1994) using the amino terminal region epitope, (KFFPGSDRFARPDFR SEQ ID NO:18).

EXAMPLE 4
Expression of Glutamate Transporter Associated Proteins

Fusion proteins and *in vitro* binding. Full-length EAAT4 was subcloned into the *EcoR* I site of the GST-fusion vector pGEX-6P-1 (Pharmacia). Synthesis of recombinant proteins in BL21 cells (Novagen) was induced by 0.1 mM isopropyl β-D-thiogalactoside for 2 hrs at 30°C and purified according to the protocol provided by the manufacturer (Pharmacia). HEK 293T cells were transfected with myc-tagged GTRAP4-41 or GTRAP4-48 and harvested in ice-cold immunoprecipitation (IP) buffer (phosphate buffered saline (pH 7.1), 5 mM EDTA, 1 mM sodium orthovanadate, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 0.3 μM aprotinin and 1% Triton X-100). The cellular lysate was incubated with GST or GST-EAAT4 immobilized on glutathione-Sepharose-4B, and washed to remove non-specifically bound proteins. Specifically bound proteins were eluted with 2 X SDS loading buffer and analyzed by immunoblotting using an anti-c-myc antibody.

The Glutathione S-transferase (GST) Gene Fusion System (Pharmacia) was used to construct and generate GST-EAAC1 and GST-GTRAP3-18 fusion proteins using pGEX-6P-1 vector as described herein.

GTRAP4-41 and 4-48 Expression Constructs. For transient expression in HEK 293T cells full-length EAAT4 cDNA was subcloned into the *Eco*R I/*Bam*H I

site of the mammalian expression vector pRK5 (Genentech). For co-immunoprecipitation full-length GTRAP4-41 and GTRAP4-48 cDNAs were subcloned into the *Not* I site of a myc-tagged pRK5 vector.

Cell culture and cell transfection. HEK 293T cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in MEM medium supplemented with 10% fetal bovine serum and L-glutamine. For transient transfections cells, were pre-washed with phosphate buffered saline (PBS) and incubated for 10 min at 4°C with 40 mg of each plasmid DNA and 20 mg of salmon sperm DNA. Cells were transfected by electroporation at 300 V and 500 μF with a gene pulser (Bio-Rad) and grown for 48-72 h in either 10 cm culture dishes or plated onto poly-D-lysine coated coverslips in 6-well plates for co-localization studies.

Subcloning, stable transfection and maintenance of cell lines The EAAT4 cDNA was subcloned into pcDNA3.1/Hygro(+) (Invitrogen) using the *EcoR* I restriction site. The plasmid was linearized with *Ssp* I, ethanol precipitated and transfected into HEK 293T cells using the calcium phosphate-DNA precipitation method. 50 mg of DNA per 10 cm dish was used. Cells were incubated with the precipitate in 5% CO_2 at 37°C for 6 hours, the medium containing the precipitate was removed and the cells were washed twice with PBS before adding fresh MEM medium. 48 h after transfection, the cells were split to 50 % confluency and hygromycin (Invitrogen) was added at a concentration of 50 mg/ml. Cell culture medium containing hygromycin was changed every 3 to 4 days. After approximately 2- to 3- weeks of selection, a serial dilution was carried out and cells were plated out, without selection, in a 96-well plate to obtain one cell per well. Several colonies were picked, expanded in selective medium and checked for expression by western blotting. Similarly, the GTRAP4-41 cDNA was cloned into pcDNA3 using the *Not* I restriction site and linearized with *Ssp* I. Selection was with G418 (Mediatech) at a concentration of 5 mg/ml. The GTRAP4-48 cDNA was cloned into the inducible expression vector pIND (Invitrogen) using the *EcoR* I restriction site and linearized with *Sca* I. Selection was with G418 and expression of GTRAP4-48 was induced with 5 mM Ponasterone A (Ecdysone- Inducible Mammalian Expression System, Invitrogen).

Co-immunoprecipitation in heterologous cells. Full-length GTRAP4-41 and GTRAP4-48 cDNAs were subcloned into the *Not* I site of a myc-tagged pRK5 vector and used to transfect the HEK-rEAAT4 cell line by electroporation at 300 V and 500 μF with a gene-pulser (Bio-Rad). After transfection (48-72h), cells were solubilized with 1 ml of ice-cold IP buffer for 2 h at 4°C with rotation and centrifuged to remove cellular debris. 5 μg of rabbit anti-NEAAT4 antibody was added to 0.5 ml of supernatant and incubated overnight at 4°C. 150μl protein A-Sepharose (Pharmacia) was then added for 2 h at 4°C, washed once with IP buffer and three times with IP minus Triton X-100. Bound protein was eluted by boiling in 3 X SDS loading buffer, and analyzed by immunoblotting using the anti-c-myc antibody.

GTRAP3-18 Expression. For transient expression in HEK 293T cells, full-length EAAC1 cDNA was subcloned into the *Eco*R I/*Bam*H I site of the mammalian expression vector pRK5 (Genentech). For co-immunoprecipitation full-length GTRAP3-18 cDNA were subcloned into the *Not* I site of a myc-tagged pRK5 vector.

Cell culture and cell transfection of GTRAP3-18 For transient transfections cells were pre-washed with PBS, incubated for with 40 mg of each plasmid DNA /20 mg of salmon sperm DNA and electroporated as described herein. In some cases, C6 glioma cells, known to naturally express high levels of EAAC1, were transfected with GTRAP3-18.

EXAMPLE 5

To determine the biochemical interaction between Glutamate Transporter Associated Proteins and glutamate transporter proteins, binding and immunoprecipitation assays *in vivo* and *in vitro* were performed.

GTRAP4-41 and 4-48 Immunoprecipitation with Fusion Proteins. Full-length EAAT4 was subcloned into the *EcoR* I site of the GST-fusion vector pGEX-6P-1 (Pharmacia). Synthesis of recombinant proteins in BL21 cells (Novagen) was induced by 0.1 mM isopropyl b-D-thiogalactoside for 2 hrs at 30°C and prepared as a crude bacterial lysate by mild sonication in ice-cold 1 C PBS and solubilization in 1% Triton X-100. Cell debris was removed by centrifugation at 7,000g and the cleared bacterial lysate applied to glutathione-Sepharose-4B (Pharmacia). HEK 293T cells were transfected with myc-tagged GTRAP4-41 or GTRAP4–48 as described herein

and harvested in ice-cold immunoprecipitation (IP) buffer (phosphate buffered saline (pH 7.1), 5 mM EDTA, 1 mM sodium orthovanadate, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 0.3 mM aprotinin and 1% Triton X-100) with 1% Triton X-100. The cellular lysate was incubated with GST or GST-EAAT4 immobilized on glutathione-Sepharose-4B, and washed to remove non-specifically bound proteins. Specifically bound proteins were eluted with 3 X SDS loading buffer and analyzed by immunoblotting using an anti-c-myc antibody. Bands were visualized by HRP-conjugated secondary antibodies and ECL chemiluminescence (Amersham).

GTRAP4-41 and GTRAP4-48 bind to GST-EAAT4 fusion protein, but do not bind to GST.

Co-immunoprecipitation in heterologous cells Transiently transfected cells (as described herein) were solubilized with 1 ml of ice-cold IP buffer for 2 h at 4°C with rotation and centrifuged to remove cellular debris. 1.2 mg of mouse anti-c-myc antibody was added to 0.5 ml of supernatant and incubated overnight at 4°C. 150 ml protein A-Sepharose (Pharmacia) was then added for 2 h at 4°C, washed once with IP buffer and three times with IP minus Triton X-100. Bound EAAT4 was eluted by boiling in 3 X SDS loading buffer, and analyzed by immunoblotting using the anti-carboxy-terminal EAAT4 antibody.

GTRAP4-41, GTRAP4-48 and KIAA0380 (a close homolog of GTRAP4-48) are coimmunoprecipitated with EAAT4 protein using the amino-terminal anti-EAAT4 antibody.

Immunoprecipitation from cerebellum lysate. Sprague-Dawley (SD) rat cerebellum was dissected, washed with 50 mM Tris-HCl (pH 7.5), 2 mM EDTA and 0.5 mM DTT, and homogenized on ice in buffer containing 20 mM Tris-HCl (pH 7.5), 10% sucrose, 1 mM EDTA, 0.1 mM PMSF, 0.3 mM aprotinin, 1 mM benamidine, 10 mg/ml leupeptine and 10 mg/ml pepstatine. Protein concentration was measured and adjusted to 2-3 mg/ml, and the homogenate was mixed in a 1:1 ratio with the solubilization buffer (homogenization buffer plus 2% Triton X-100). After 2 h, the lysate was spun at 10,000g for 10 min. For each immunoprecipitation, 500 mg of the Triton-lysate was incubated overnight at 4°C with 5 μg of the anti-amino-terminal EAAT4 antibody. Immune complexes were precipitated with protein

Virus was harvested about 72 hrs after transfection and titered as previously described (Bowers *et al. Mol. Ther.* **1**, 294-299 (2000)). 2 x 10^5 expression particles were injected intra-cisternally into male Sprague-Dawley rats (250 g) obtained from Zivic Miller. About 48 h after injection, the rats were sacrificed and synaptosomal preparations of the cerebelli were prepared using a polytron. Glutamate transport was measured by methods described herein.

GTRAP4-41 and GTRAP4-48 produce a two- to four-fold increase in glutamate transport, respectively. The co-expression of GTRAP4-41 and GTRAP4-48 results in a further increase in glutamate uptake. Kinetic analysis indicates that GTRAP4-41 and GTRAP4-48 produced an increase in the V_{max} of glutamate transport activity (Fig. 3). There is also a small increase in the K_m values when GTRAP4-41 and GTRAP4-48 are co-expressed, but these are not statistically significant, suggesting that the interacting proteins do not alter the affinity of the transporter for glutamate. GTRAP4-41 and GTRAP4-48 may therefore enhance glutamate transport either via an increase in the catalytic rate of the transporter or via an increase in cell surface availability. Results are presented in Figure 3. GTRAP41 and GTRAP48 expression in HEK-rEAAT4 cells increase glutamate uptake significantly over vector alone (VA) transfected cells. Data in Fig. 3A are the mean ± SEM of at least four independent observations and were compared by students *t* test, (** $p < 0.005$). Concentration dependence of Na^+-dependent L-[^{3}H]-glutamate uptake was assayed in the presence of increasing concentrations of glutamate. In Fig. 3B, the values are expressed as the mean ± SEM of a representative experiment carried out in triplicate. Kinetic data shows that GTRAP41 (▯) increases the V_{max} from 222 to 605 pmol/mg/min and increases the K_m slightly from 7 to 11 μM, compared to EAAT4 alone (▲). GTRAP48 increases the V_{max} from 208 to 512 pmol/mg/min (●) and increased the K_m from 10 to 13 μM.

To test if GTRAP3-18 modulates EAAC1 function, sodium-dependent [^{3}H]-glutamate transport was studied in HEK293 cells co-expressing both proteins, 72 hrs after transfection (Rothstein *et al. Neuron* **16**, 675-686 (1996); Lin *et al., Neuron* **20**, 589-602 (1998)). Total glutamate transport progressively decreases with increasing GTRAP3-18 protein expression (Fig. 4). GTRAP3-18 negatively modulates EAAC1-mediated glutamate transport. Glutamate transport was studied in HEK293 cells transfected with plasmids indicated in Fig . 4 . GTRAP3-18 inhibited EAAC1-

mediated transport, but had no effect on EAAT4 (n=6). The co-expression of GTRAP-3-18 has no effect on total EAAC1 protein expression. Analysis of HEK293 cells by confocal microscopy and surface biotinylation reveal no alteration in the membranous localization of EAAC1. Superoxide dismutase (SOD1) was used as a control. Eadie-Scatchard plot of glutamate transport in transfected HEK293 cells reveals a 4-10 fold decrease in affinity (n=4). This effect is specific for EAAC1; co-expression of GTRAP3-18 with EAAT4 has no effect on transport activity. The inhibition of transport is not due to a decrease of EAAC1 protein level by the co-expression of GTRAP3-18, as quantitated by Western blotting. Similarly, the loss of EAAC1 activity is not due to altered protein trafficking; even at high levels of GTRAP3-18 expression, when little EAAC1-mediated transport is observed, EAAC1 surface expression is unaltered as determined by surface biotinylation and confocal microscopy.

To evaluate the biochemical nature of altered transport, kinetic analyses were performed with HEK293 cells co-expressing EAAC1 and GTRAP3-18. EAAC1 and GTRAP3-18 co-expressing cells show a decrease in affinity (K_m =40 μM, Vmax= 0.99 nmol/min/mg protein; n=4, P<0.01) without a shift in the V_{max} when compared to cells only expressing EAAC1 (K_m =9 μM; V_{max}=1.02 nmol/min/mg protein; Figure 4A). Similar results are observed with other cell lines (COS7 and C6 glioma).

EXAMPLE 9

Cell Surface Levels of GTRAPs and Cytoskeletal Stability

To examine changes in the cell surface levels of EAAT4, a cell membrane-impermeant biotinylation reagent to label cell surface proteins selectively. Biotinylation of cell surface proteins was performed as described in Duan et al. (Duan, *et al., J. Neurosci.* **19**, 10193-10200 (1999)). SOD1 was used to control for total protein and to determine whether the biotinylation reagent labels proteins in the intracellular compartment. Densitometry was performed using the NIH Image program.

The total amount of EAAT4 is increased when GTRAP4-41 and GTRAP4-48 are co-expressed (Fig. 5). In contrast the total amount of SOD1, a control for total amount of protein loaded, is unaltered or decreased in the GTRAP4-41 and GTRAP4-48 samples, respectively. The majority of the EAAT4 is biotinylated, indicating that it is at the cell surface. However the percentage of total EAAT4 that is at the cell

surface remains the same when GTRAP4-41 and GTRAP4-48 are co-expressed. Taken together, these results indicate that GTRAP4-41 and GTRAP4-48 stabilize/anchor EAAT4 at the cell membrane, making it less likely to be internalized and subsequently degraded, rather than causing an increased trafficking of EAAT4 to the cell surface.

However it is also possible that there is increased expression of the cell's native gene. To address this question cells were treated 48 hrs after transfection with cycloheximide, an inhibitor of protein synthesis. Quantification by densitometry shows that 12 hrs after treatment, the EAAT4 protein in HEK-rEAAT4 cells is reduced to 54 ± 0.6% of the level prior to cycloheximide treatment. In contrast, 81 ± 2% and 74 ± 1.7% of the EAAT4 protein remains after 12 hrs when GTRAP4-41 and GTRAP4-48 are coexpressed, respectively. These results provide evidence that GTRAP4-41 and GTRAP4-48 do stabilize EAAT4 at the membrane.

EXAMPLE 10

GTRAP- Glutamate Transport Protein Interactions

To determine whether the EAAT4/GTRAP4-48 interaction is required to mediate the increase in EAAT4 activity, HEK-rEAAT4 cells were transfected with GTRAP4-48 constructs lacking the last 155 amino acids which were pulled out by EAAT4 in the yeast two-hybrid screen . The carboxy-terminally truncated GTRAP4-48 had only a modest effect on stimulating EAAT4 activity, indicating that the protein-protein interaction is responsible for the majority of the increase in uptake activity. HEK-rEAAT4 cells were co-transfected with GTRAP4-48 and a myc-tagged cDNA construct encoding the last 77 amino acids of EAAT4 to disrupt the interaction of GTRAP4-48 with full-length EAAT4. Co-expression of this construct inhibits the GTRAP4-48 mediated effect by approximately 25%, but co-expression of a smaller construct (residues 1452 to 1578), which lacked the GTRAP4-48 binding domain, has no effect. Taken together these results indicate that the EAAT4/GTRAP4-48 interaction plays an important role in the modulation of EAAT4 uptake activity.

These results are summarized in Figure 6. Fig 6A shows results of experiments in which HEK-rEAAT4 cells were transfected with a GTRAP48 construct that lacked the C-terminus (domain that interacts with EAAT4). Disruption of the EAAT4/GTRAP48 interaction significantly reduces the GTRAP48-mediated increase in EAAT4 uptake activity (* $p< 0.05$). Disruption of the protein-protein

interaction by overexpression of the EAAT4 C-terminus in HEK-rEAAT4 cells transfected with GTRAP48. The GTRAP48-mediated effect on EAAT4 activity was reduced by ~25% (** $p< 0.005$; Fig 6B). Na^+-dependent L-[^{3}H]-glutamate was assayed in triplicate and values are expressed as the mean ± SEM of six independent experiments. GTRAP41 and GTRAP48 significantly increased glutamate uptake *in vivo* (* $p < 0.05$; Fig 6C) .

The physiological relevance of GTRAP4-41 and GTRAP4-48 on EAAT4 uptake activity *in vivo* was subsequently examined by the intra-cisternal injection of HSV amplicon vectors expressing GTRAP4-41 and GTRAP4-48. Cerebellar glutamate uptake was measured 48 hrs after injection and found to be elevated when GTRAP4-41 and GTRAP4-48 are expressed but not when the control HSVlac amplicon vector was injected (Fig. 6C). Dihydrokainic acid (DHK), an inhibitor of GLT-1 mediated glutamate transport, has no effect on cerebellar glutamate uptake, ruling out any involvement of GLT-1. Unfortunately there is no method to distinguish functionally between GLAST, EAAC1 and EAAT4 . However it has been have shown that GTRAP4-41 and GTRAP4-48 do not interact directly with any other transporter, it is likely that the observed increase in uptake is attributed to an increase in EAAT4 activity. Western blot analysis confirms increased expression of GTRAP4-41 and GTRAP4-48 in the cerebellum following the injections.

EXAMPLE 11

Clustering of Glutamate Transporter Proteins at Synapses

To examine whether GTRAPs are involved in, or associated with, the clustering of EAAT4 at synapses primary cultures of rat Purkinje cell neurons were examined immunocytochemically. Rat brain sections were stained, as previously described (Furuta *et al.,* Neurosciences 81:1031-1042 (1997)) using the following antibodies: carboxy-terminal anti-EAAT4 (1.5 μg/ml), anti-GTRAP4-41 (127 ng/ml) or anti-GTRAP4-48 (132 ng/ml) antibodies. Texas-red and FITC-conjugated secondary antibodies were used at dilutions of 1:200.

EAAT4 and GTRAP4-41 immunoreactivity is observed throughout the soma and dendrites but is also found to colocalize in distinct clusters. Labeling with synaptophysin, a presynaptic protein, reveals that 71% of synapses possessed clusters of EAAT4 and GTRAP4-41 [n=12]. This perisynaptic distribution of GTRAP4-41 correlates with earlier EM studies that showed that EAAT4 is a perisynaptic protein.

Similar studies could not be carried out for GTRAP4-48 due to low level of expression at this early developmental stage.

EXAMPLE 12

Interaction with Rho: Since GTRAP4-48 possesses areas of homology to p115 and PDZRhoGEF, two novel RhoGEFs that selectively activate Rho (Hart *et al., J. Biol. Chem.* **271**, 25452-25458 (1996), Fukuhara *et al., J. Biol. Chem.* **274**, 5868-5879 (1999)), interaction of GTRAP4-48 with the Rho family of GTPases was investigated.

Guanine nucleotide exchange assay. Small G proteins GST-RhoA, GST-CDC42 and GST-Rac were expressed in bacterial cells and affinity purified to ~80% purity using a glutathione column. Twenty pmoles of each protein were incubated with 100 pmoles GTPγS for 10 min at 30°C with varying concentrations of full-length GTRAP4-48 or p115. The binding reactions were filtered through BA-85 nitrocellulose and the amount of GTPγS bound to small G protein was quantitated by scintillation counting of the dried filters. The amount of GTPγS that bound to GST-RhoA, GST-Cdc42 and GST-Rac in the presence of full-length GTRAP4-48 or p115 was measured.

GTRAP4-48, like p115, demonstrates a specific guanine nucleotide exchange activity for Rho (Fig. 7). Co-immunoprecipitation assays also show that GTRAP4-48 interacts with the active form (in the presence of aluminium fluoride) of the $G\alpha_{13}$ subunit and therefore, may act as a link between G-protein coupled receptors and their downstream targets. However, unlike p115, regulation of the GTRAP4-48 RhoGEF activity by $G\alpha_{13}$ nor the stimulation of the GTPase activity of $G\alpha_{13}$ by GTRAP4-48 could be demonstrated. Rho is known to regulate the remodeling of the actin cytoskeleton through various actin-binding proteins, although the mechanism is not yet well characterized (Hall, *Science* **279**, 509-514 (1998)).

Since GTRAP4-48 can activate Rho, expression of GTRAP4-48 was studied to determine if it could induce the reorganization of the actin cytoskeleton and whether it alters the distribution of GTRAP4-41, a possible actin binding protein. When GTRAP4-41 is expressed alone there is a close relationship between actin and GTRAP4-41 at the cell membrane but there are very few organized actin filaments. Conversely, when GTRAP4-41 and GTRAP4-48 are co-expressed, GTRAP4-41 is

found to co-localize with actin in structures that resembled actin-stress fibers, a typical Rho-dependent effect. Overexpression of GTRAP4-48 also induces the formation of membrane ruffling and filopodia, suggesting some degree of cross-talk between the small GTPases, as these are typical Rac and Cdc42 dependent effects. These results indicate that there is a close relationship between GTRAP4-48 and the reorganization of GTRAP4-41 and the actin cytoskeleton.

EXAMPLE 13

Antisense Treatment with GTRAP3-18

To demonstrate tonic modulation of EAAC1 activity by GTRAP3-18, antisense oligomers were used to lower GTRAP3-18 expression in HEK293 cells. Western blot analyses and glutamate uptake assays reveal endogenous expression of EAAC1 and GTRAP3-18 protein in HEK cells, but no expression of other transporter subtypes, *e.g.,* GLAST, GLT-1, or EAAT4. Antisense oligomers, targeted to the 5'-GTRAP3-18 transcript, were transfected into HEK293 cells.

Antisense oligomers specifically reduced endogenous GTRAP3-18 protein level (Fig. 8A, gray bars); EAAC1 protein level was not affected. Significantly, glutamate transport activity was concomitantly elevated with the reduction of GTRAP3-18 protein level (black bars).

To examine modulation of EAAC1 by GTRAP3-18 *in vivo*, GTRAP3-18 antisense oligomers were administered intraventricularly. Sequences for the novel phosphodiester oligonucleotides used were: sense GTRAP3-18: 5'-GTGAACCTTGCCCGCTC-3', antisense GTRAP3-18: 5'-GAGCGGGGCAAGGTTCAC-3' Oligonucleotides (5μg/μL), separately or in combination were administered intraventricularly over 3-11 days, by mini-osmotic pumps (Alza Corp., Palo Alto, CA) as described previously (Rothstein *et al.,* (1994)).

Eleven days of antisense treatment resulted in a reduction of GTRAP3-18 protein level and a significant increase in cortical glutamate uptake, whereas glutamate uptake was not altered in sense oligomer-treated animals (Figure 8B). The effect was due to increased EAAC1-mediated transport because it was not altered by dihydrokainic acid (DHK), an inhibitor of GLT-1-mediated glutamate transport (Robinson *et al. (1998)* Neurochem. Int. 33:479-491). In kinetic studies of DHK-insensitive, cortical glutamate uptake from antisense-treated animals, the apparent

affinity for glutamate was increased (antisense K_m=10μM, V_{max}=1.08 nmol/min/mg protein) compared to artifical CSF or sense treated control animals (control K_m=19.7 μM; V_{max}= 1.08 nmol/min/mg protein; Figure 8C). These results suggest that GTRAP3-18 negatively modulates EAAC1 glutamate transport activity in vivo.

EXAMPLE 14

Regulation of GTRAP3-18 by Retinoic Acid

Human GTRAP3-18 (JWA protein) was originally identified as a retinoic acid responsive gene. Therefore, retinoic acid was tested for its ability to up-regulate GTRAP3-18 expression and consequently inhibit EAAC1-mediated glutamate transport in HEK293 cells. Retinoic acid induces a large increase in GTRAP3-18 expression, over a non-toxic dose range from 1-10 μM. A significant decrease in glutamate transport activity paralleled the increase of GTRAP3-18 protein level (Figure 9). The loss of transport activity is not due to changes in EAAC1 protein level (Figure 9A) or the cellular membrane localization of EAAC1 protein by retinoic acid as examined by fluorescent microscopy. To confirm that loss of transport activity was specifically due to GTRAP3-18 and not by other factors induced by retinoic acid or direct effects on EAAC1, a truncated EAAC1 cDNA, lacking the last 93 amino acids, was constructed. The truncation corresponded to the region used as bait in yeast two-hybrid screening, and was not able to interact with GTRAP3-18. Nevertheless, after transient expression in HEK293 cells, the truncated EAAC1 transported glutamate. Importantly, retinoic acid treatment does not alter activity of the truncated EAAC1 protein; even though GTRAP3-18 protein expression was markedly elevated (Figure10B). Thus, the loss of transport activity by retinoic acid was the result of GTRAP3-18 induction. Interestingly, truncated EAAC1 has increased glutamate transport activity compared to wild-type. Truncated EAAC1 had a K_m of 5.4 μM, which was greater than a three-fold increase in affinity compared to wild-type EAAC1 (Km=17 μM; Figure 9C). This could reflect lack of natural inhibition of the truncated protein EAAC1 by endogenous GTRAP3-18 – results similar to the effects of GTRAP3-18 antisense treatment (Figure 9E).

To test this hypothesis in vivo, retinoic acid was infused intraventricularly (1-20μM; 0-20 pmol/μL). After 4 days of treatment, cortical GTRAP3-18 protein expression was increased in a dose dependent manner, and this was associated with a

significant decrease of total glutamate uptake (Figure 9D, top panel). This effect is specifically due to decreased EAAC1-mediated transport because it was not altered by the glutamate transport inhibitor dihydrokainic acid, at a concentration that predominantly effects GLT-1 (Robinson *et al.* (1998)). Kinetic analysis of DHK-insensitive, cortical glutamate transport from animals treated four days with intraventricular retinoic acid reveals a 4-fold decrease in affinity compared to control transport (Figure 9E) which is very similar to that seen in vitro (Figure 9B). In addition, retinoic acid inhibition of glutamate transport could be reversed in vivo; chronic intraventricular treatment with antisense GTRAP3-18 oligomer (50-100 ng/day, for 7-10 days) blocks the retinoic acid (2.5 μM) induction of GTRAP3-18, and also blocks the inhibition of glutamate transport seen with retinoic acid treatment (Figure 9D, top panel). Retinoic acid had no effect on glutamate transport by cells expressing GLT-1 or EAAT4.

EXAMPLE 15

Glutamate Transporter Associated Protein PCTAIRE-1

The glutamate transporter EAAT4 possesses high affinity Na^+-dependent transport activity, as well as a unique ligand-gated Cl^- conductance. Largely located in the somatodendritic compartment of the cerebellar Purkinje cell, altered function of EAAT4 may contribute to the pathogenesis of spinocerebellar ataxia and alcoholic cerebellar degeneration. In an effort to delineate possible regulatory mechanisms of EAAT4, we have identified glutamate transporter associated proteins (GTRAPs). Using the amino terminus of rat EAAT4 as bait in a yeast two-hybrid screen, an interacting protein was isolated. Subsequent sequence analysis identified the GTRAP as PCTAIRE-1, a serine/threonine kinase related to the cyclin-dependent kinase 2 (cdk2) family. In vitro and in vivo co-immunoprecipitations from rat cerebelli were performed, confirming specificity of interaction; co-localization of EAAT4 and PCTAIRE-1 within the cerebellum was determined using immunofluorescence. In order to investigate regulatory physiology of the PCTAIRE-1/EAAT4 interaction, co-transfection experiments and pharmacologic manipulation were carried out. PCTAIRE-1, although a member of the cdk2 family, is present mainly in terminally differentiated tissues such as brain. It has been shown to interact with members of signal transduction cascades (14-3-3 proteins) and components of cellular protein networks such as p11, a target for annexin II. These data suggest a mechanism by

which EAAT4 may be linked to cellular regulatory machinery via the GTRAP PCTAIRE-1.

Methods. Yeast Two-Hybrid Screening: Screening was performed using the AH109 yeast strain harboring the reporter genes ADE/HIS, as well α- and β-galactosidase. The initial 60 amino acids of EAAT4 were subcloned in-frame into pGBKT7 (GAL4 binding domain, CLONTECH), and used to screen a rat brain cDNA library constructed in pACT2 (GAL4 activation domain, CLONTECH). Following cotransformation and verification of true positive colonies, DNA sequence analysis was performed. Obtained sequences were compared to known GENBANK submissions, resulting in identification of a true postive with with >95% homology to the final 201 amino acids of rat PCTAIRE-1.

Creation of expression constructs: Full length rat PCTAIRE-1 was isolated from a rat brain cDNA library via PCR amplification using upstream and downstream primers based on the known PCTAIRE sequence. Products were cloned into pCMVmyc tagged eukaryotic expression vector (CLONTECH), and expression verified by western blotting.

Two PCTAIRE-1 proteins are identified. PCTAIRE-1a is encoded by PCTAIRE-1 nucleic acid sequence, nucleotides 251-452 and 584-1872 (SEQ ID NO:8) and PCTAIRE-1b is encoded by PCTAIRE-1 nucleic acid sequence, nucleotides 487-1872 (SEQ ID NO:22).

Immunoprecipitations. In-vitro coimmunoprecipitaions were performed on stably transfected HEK cells expressing EAAT4. Vector DNA or myc-tagged PCTAIRE vector was then introduced. Following expression, cells were solubilized with ice-cold IP buffer and centrifuged to remove cellular debris. 0.5 ml of supernatant was then incubated with or without 1.5 μg anti-c-myc antibody (Boehringer-Mannheim). Complexes were then isolated using protein-A Sepharose, washed four times with IP buffer with and without Triton X-100, and visualized using SDS-PAGE. EAAT4 western blotting was performed using affinity purified rabbit polyclonal Ab at 1:200 dilution.

In-vitro coimmunoprecipitation of EAAT4 with myc tagged antibody in EAAT4 expressing HEK cells transfected with myc-labeled PCTAIREs is demonstrated.

In-vivo coimmunoprecipitation was performed using the cerebellum of a 5 day-old Sprague-Dawley rat. Homogenization was performed on ice using a buffer containing 20 MM Tris-HCl (pH 7.5), 10% sucrose, 1 mM EDTA, and protease inhibitors. The homogenate was mixed 1:1 with buffer containing 2% Triton X-100, and solubilized for 2 h at 4°C. 0.5 mg of protein was used for each immunoprecipitation. Antibodies to the carboxy terminal EAAT4 (2.0 μg), as well as antibody to the transporter GLT (2.0 μg) were used. In addition, blocking peptide was presorbed to EAAT4 Ab to further demonstrate specificity. Western blotting was performed using PCTAIRE-1 antibody at 1:200 dilution (Santa Cruz).

In-vivo coimmunoprecipitation of PCTAIRE by EAAT4 is found in neonatal rat cerebellum. A PCTAIRE doublet (62 and 68 kDa) is recovered by immunoprecipitation with c-terminal EAAT4 Ab, and inhibited by preabsorption of EAAT4 Ab with blocking peptide.

Transfection of EAAT4 expressing HEK cells with PCTAIRE results in diminished Na^+-dependent glutamate uptake. HEK cells and EAAT4 expressing HEK cells were transfected with 1.0 μg of pCMV PCTAIRE plasmid, and allowed to express for 48 hours. Cell monolayers were then washed with tissue buffer (50 mM Tris, 320 mM sucrose, pH 7.4). The cells were then incubated for 4 min at 37°C with 1 mL of either sodium-(120 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 2 mM $CaCl_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 10% glucose and 10 μM glutamate or choline-(120 mM choline-Cl, 25 mM Tris-HCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 10% glucose and 10 μM glutamate) containing buffer. Glutamate uptake assays were then performed using L-[^{3}H]-Glutamate in the presence and absence of Na^+. After rinsing, cells were lysed in 0.1 N NaOH and lysate radioactivity measured using a scintillation counter. Protein content was measured and glutamate uptake calculated as the difference between Na^+ containing and sodium free values per mg of protein.

Inhibition of Na^+-dependent glutamate uptake by PCTAIRE is reversible using the cdk2 inhibitor olomucine. HEK cells expressing EAAT4 were transfected with 1.0 μg of pCMV PCTAIRE plasmid as described above, and allowed to express for 48 hours. Prior to glutamate uptake assay, cells were treated with 100 μM olomucine for 30 minutes at 37°C as indicated. Olomocine belongs to a class of cyclin

dependent kinase inhibitors which inhibit activity via competition at the ATP binding site.

Immunofluorescence microscopy displays colocalization of EAAT4 and PCTAIRE in the Purkinje cell layer of the rat cerebellum. A five day-old rat pup was perfusion fixed, the brain extracted, and 25 μm sections stained with antibodies to c-terminal EAAT4 (1.5μg/mL) and PCTAIRE-1 (1.5μg/mL). Prominent double-labeling is evident in the Purkinje cell layer, especially the cell soma, where EAAT4 is known to be present during the early postnatal period.

These results indicate that the serine/threonine kinase PCTAIRE interacts with the amino-terminus of the glutamate transporter EAAT4. This interaction results in downregulation of Na^+-dependent glutamate uptake, and this process is reversible using an inhibitor of cyclin dependent kinases. In addition, immunofluorescence reveals that both EAAT4 and PCTAIRE localize to the cerebellum, particularly the purkinje cell layer. Although PCTAIRE bears homology to the family of cyclin dependent kinases involved in proliferation, it is found mainly in terminally differentiated tissues such as brain. Other EAAT4 interacting proteins have recently been identified, both of which interact at the carboxy-terminus, and upregulate glutamate uptake. GTRAP 41 is a new member of the β-III spectrin family, and is likely an actin-binding protein. GTRAP4-48 is a novel RhoGEF that may provide a link between the heterotrimeric G-proteins and small GTP-binding proteins of the Rho family. Together with PCTAIRE, these interactors may regulate glutamate uptake through EAAT4.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. A substantially pure polypeptide characterized as:
   (a) modulating intracellular glutamate transport;
   (b) interacting with a glutamate transporter protein; and
   (c) having an expression pattern in the brain.

2. The polypeptide of claim 1, wherein the glutamate transporter protein is EAAT4.

3. The polypeptide of claim 1, wherein the modulation is an increase in glutamate transport.

4. The polypeptide of claim 1, wherein the polypeptide is further characterized as:
   (a) having at least one PDZ domain;
   (b) having at least one regulatory G-protein domain;
   (c) having at least one pleckstrin homology domain;
   (d) having at least one proline-rich domain; and
   (e) having at least one guanine exchange factor domain.

5. The polypeptide of claim 1, wherein the polypeptide is further characterized by
   (a) having at least one pleckstrin homology domain;
   (b) having at least one spectrin repeat; and
   (c) having at least one α-actinin domain.

6. The polypeptide of claim 1, wherein the expression pattern is in Purkinje cells.

7. The polypeptide of claim 1, wherein the polypeptide has an amino acid sequence as set forth in SEQ ID NO:2.

8. The polypeptide of claim 1, wherein the polypeptide has an amino acid sequence as set forth in SEQ ID NO:4.

9. A substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:2, or conservative variants thereof.

10. A substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:4, or conservative variants thereof.

11. An isolated polynucleotide encoding a polypeptide according to claim 1.

12. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:2;

(b) a polynucleotide of (a), wherein T can be U;

(c) a polynucleotide complementary to (a) or (b);

(d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1;

(e) degenerate variants of (a), (b), (c) or (d); and

(f) a fragment of (a), (b), (c), (d) or (e) having at least 15 base pairs and hybridizes to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:2.

13. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:4;

(b) a polynucleotide of (a), wherein T can be U;

(c) a polynucleotide complementary to (a) or (b);

(d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:3;

(e) degenerate variants of (a), (b), (c) or (d); and

(f) a fragment of (a), (b), (c), (d) or (e) having at least 15 base pairs and hybridizes to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:4.

14. An antibody that binds to a polypeptide of claim 7 or 8 or binds to immunoreactive fragments thereof.

15. The antibody of claim 14, wherein the antibody is polyclonal.

16. The antibody of claim 14, wherein the antibody is monoclonal.

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17. The antibody of claim 14, wherein the antibody disrupts interaction of the polypeptide with a glutamate transporter protein.

18. An expression vector comprising a polynucleotide of claim 11.

19. The expression vector of claim 18, wherein the vector is virus-derived.

20. The expression vector of claim 18, wherein the vector is plasmid-derived.

21. A host cell comprising a vector of claim 18.

22. A method for producing a polypeptide comprising the steps of:

(a) culturing a host cell of claim 21 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

23. A substantially pure polypeptide, wherein the polypeptide interacts with amino acid sequence QEAELTLP (SEQ ID NO:9) or amino acid sequence GRGGNESVM (SEQ ID NO:10).

24. A polynucleotide encoding the polypeptide of claim 23.

25. A substantially pure polypeptide, wherein the polypeptide interacts with the amino acid sequence set forth in SEQ ID NO:12.

26. A substantially pure polypeptide, wherein the polypeptide interacts with the amino acid sequence set forth in SEQ ID NO:13.

27. A method for identifying a compound that modulates a cellular response mediated by a Glutamate Transporter Associated Protein comprising:

(a) incubating the compound with a cell expressing a Glutamate Transporter Associated Protein and a glutamate transporter protein under conditions sufficient to permit the components to interact

(a) comparing a cellular response in the cell incubated with the compound with the cellular response of a cell not incubated with the compound;

thereby identifying a compound that modulates a cellular response mediated by a Glutamate Transporter Associated Protein .

28. The method of claim 27, wherein the cellular response is an increase in glutamate transport.

29. The method of claim 27, wherein the cellular response is a decrease in glutamate transport.

30. The method of claim 27, wherein the cellular response is an increase in cytoskeletal stability.

31. The method of claim 27, wherein the cellular response is a decrease in cytoskeletal stability.

32. The method of claim 27, wherein the cellular response is an increase in chloride flux.

33. The method of claim 27, wherein the cellular response is a decrease in chloride flux.

34. The method of claim 27, wherein the Glutamate Transporter Associated Protein is selected from the group consisting of GTRAP4-41, GTRAP4-48, PCTAIRE-1 and GTRAP3-18.

35. The method of claim 27, wherein the glutamate transport protein is selected from the group consisting of GLAST, GLT-1, EAAC1, EAAT1, EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5.

36. The method of claim 27, wherein the glutamate transport protein is EAAT4 and the Glutamate Transporter Associated Protein is GTRAP4-41, GTRAP4-48 or PCTAIRE-1.

37. The method of claim 27, wherein the glutamate transport protein is EAAC1 and the Glutamate Transporter Associated Protein is GTRAP3-18.

38. The method of claim 27, wherein the cell further expresses a RhoGEF protein.

39. The method of claim 27, wherein the compound is selected from the species consisting of a peptide, a peptidomimetic, a polypeptide, a pharmaceutical, a chemical compound, a biological agent and an antibody.

40. The method of claim 27, wherein the cell is selected from the group consisting of a neuronal cell, a glial cell, a cardiac cell, a bronchial cell, a uterine cell, a testicular cell, a liver cell, a renal cell, an intestinal cell, a thymus cell, a spleen cell, a placental cell, a skeletal muscle cell and a smooth muscle cell.

41. A method for identifying a compound that inhibits an interaction between a Glutamate Transporter Associated Protein and a glutamate transporter protein comprising:

(a) contacting a Glutamate Transporter Associated Protein with a glutamate transporter protein in the presence of the compound and

(b) comparing the formation of a Glutamate Transporter Associated Protein-glutamate transporter protein complex in the presence of the compound with a formation of the complex in the absence of the compound,

thereby identifying a compound that inhibits an interaction between a Glutamate Transporter Associated Protein and a glutamate transporter protein.

42. The method of claim 41, wherein the Glutamate Transporter Associated Protein is selected from the group consisting of GTRAP4-41, GTRAP4-48, PCTAIRE-1 and GTRAP3-18.

43. The method of claim 41, wherein the glutamate transport protein is selected from the group consisting of GLAST, GLT-1, EAAC1, EAAT1, EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5.

44. The method of claim 41, wherein the glutamate transport protein is EAAT4 and the Glutamate Transporter Associated Protein is GTRAP4-41, GTRAP4-48 or PCTAIRE-1.

45. A method of treating a disorder associated with glutamate transport comprising administering to a subject in need thereof a therapeutically effective amount of a compound that modulates a Glutamate Transporter Associated Protein activity or interaction with a glutamate transporter protein.

46. The method of claim 45, wherein the Glutamate Transporter Associated Protein is GTRAP4-41, GTRAP4-48 or PCTAIRE-1 .

47. The method of claim 46, wherein the disorder is a disorder of the nervous system.

48. The method of claim 47, wherein the disorder of the nervous system is neurodegeneration, Spinocerebellar ataxia type 1 (SCA1), or schizophrenia.

49. The method of claim 45, wherein the Glutamate Transporter Associated Protein is GTRAP3-18.

50. The method of claim 49, wherein the disorder is epilepsy or a disorder of GABA metabolism or schizophrenia.

51. The method of claim 45, wherein the compound is selected from the species consisting of a peptide, a peptidomimetic, a polypeptide, a pharmaceutical, a chemical compound, a biological agent and an antibody.

52. A method of treating a disorder associated with chloride flux comprising administering to a subject in need thereof a therapeutically effective amount of a compound that modulates a Glutamate Transporter Associated Protein activity or interaction with a glutamate transporter protein.

53. The method of claim 52, wherein the Glutamate Transporter Associated Protein is GTRAP4-41, GTRAP4-48, PCTAIRE-1 or GTRAP3-18.

54. The method of claim 52, wherein the disorder is a disorder of the nervous system.

55. A method of modulating glutamate transport in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound that modulates expression of a Glutamate Transporter Associated Protein, thereby modulating glutamate transport.

56. The method of claim 55, wherein the Glutamate Transporter Associated Protein is GTRAP3-18.

57. The method of claim 55, wherein the compound is a polynucleotide having a nucleic acid sequence substantially similar to SEQ ID NO:20. (5'-GAGCGGGGCAAGGTTCAC-3')

58. The method of claim 55, wherein the compound is retinoic acid.

59. The method of claim 56, wherein the modulation of glutamate transport is an decrease in glutamate transport.

60. The method of claim 55, wherein the Glutamate Transporter Associated Protein is GTRAP4-41, PCTAIRE-1 or GTRAP4-48.

61. The method of claim 60, wherein the modulation of glutamate transport is a increase in glutamate transport.

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62. A transgenic non-human animal having a transgene that expresses a polypeptide of claim 1 chromosomally integrated into the germ cells of the animal.

63. The transgenic animal of claim 62, wherein the animal is murine.

64. A transgenic non-human animal whose genome comprises a disruption of a Glutamate Transporter Associated Protein gene, wherein the disruption comprises the insertion of a transgene comprising a selectable marker sequence, and wherein the disruption results in a disorder of the nervous system as compared to a wild-type animal not having the disruption.

65. A transgenic animal according to claim 64, wherein the disorder of the nervous system is selected from the group consisting of a disorder of glutamate transport, neurodegeneration, epilepsy, glutamate toxicity, disorders of memory, disorders of learning, Alzheimer's disease, cerebellar degenerative disease, spinocerebellar ataxia type 1, Huntington's disease, perinatal hypoxia, amyotrophic lateral sclerosis and alcoholic brain injury.

66. A transgenic animal of claim 64, wherein said animal is heterozygous or homozygous for the disruption of the endogenous Glutamate Transporter Associated Protein gene.

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67. A method for producing a transgenic mouse exhibiting a disorder of the nervous system, said method comprising:

(a) introducing a transgene comprising a selectable marker sequence into a mouse embryonic stem cell;

(b) introducing the mouse embryonic stem cell into a mouse embryo;

(c) transplanting the embryo into a pseudopregnant mouse; allowing the embryo to develop to term; and

(d) identifying a transgenic mouse whose genome comprises a disruption of the endogenous Glutamate Transporter Associated Protein gene, wherein the disruption results in the mouse exhibiting a disorder of the nervous system as compared to a wild-type mouse.

68. A method according to claim 67, wherein the transgenic mouse is homozygous for the disruption of the endogenous Glutamate Transporter Associated Protein gene.

69. A method according to claim 67, wherein the transgenic mouse is heterozygous for the disruption of the endogenous Glutamate Transporter Associated Protein gene.

70. A computer readable medium having stored thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and sequences substantially identical thereto.

71. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and sequences substantially identical thereto.

72. The computer system of claim 71, further comprising a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon.

73. The computer system of claim 71, wherein the sequence comparison algorithm comprises a computer program which indicates polymorphisms.

74. The computer system of claim 71, further comprising an identifier which identifies features in said sequence.

75. A method for comparing a first sequence to a reference sequence wherein said first sequence is a nucleic acid sequence selected from the group consisting SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and sequences substantially identical thereto comprising:

(a) reading the first sequence and the reference sequence through use of a computer program which compares sequences; and

(b) determining differences between the first sequence and the reference sequence with the computer program.

76. The method of claim 75, wherein determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.

77. A method for identifying a feature in a sequence wherein the sequence is selected from the group consisting of a nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, sequences substantially identical thereto, or a polypeptide sequence SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6and sequences substantially identical thereto comprising:

(a) reading the sequence through the use of a computer program which identifies features in sequences; and

(b) identifying features in the sequences with the computer program.

78. A substantially pure polypeptide characterized as:

(a) modulating intracellular glutamate transport;

(b) interacting with a glutamate transporter protein;

(c) having an expression pattern in neural non-neuronal tissues;

(d) having at least one kinase C domains;

(e) having four transmembrane domains; and

(f) being hydrophobic.

79. The polypeptide of claim 78, wherein the glutamate transporter protein is EAAC1.

80. The polypeptide of claim 78, wherein the modulation is a decrease in glutamate transport.

81. The polypeptide of claim 78, wherein the polypeptide has an amino acid sequence as set forth in SEQ ID NO:6.

82. A substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:6, or conservative variants thereof.

83. An isolated polynucleotide encoding a polypeptide according to claim 78.

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84. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:6;

(b) a polynucleotide of (a), wherein T can be U;

(c) a polynucleotide complementary to (a) or (b);

(d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:5;

(e) degenerate variants of (a), (b), (c) or (d); and

(f) a fragment of (a), (b), (c), (d) or (e) having at least 15 base pairs and hybridizes to a polynucleotide encoding a polypeptide as set forth in SEQ ID NO:6.

85. An antibody that binds to a polypeptide of claim 82 or binds to immunoreactive fragments thereof.

86. The antibody of claim 85, wherein the antibody is polyclonal.

87. The antibody of claim 85, wherein the antibody is monoclonal.

88. The antibody of claim 85, wherein the antibody disrupts interaction of the polypeptide with a glutamate transporter protein.

DRAFT - 12/05/2005

ABSTRACT

Glutamate Transporter Associated Proteins and nucleotide encoding Glutamate Transporter Associated Proteins are provided. Also provided is a method for identifying a compound that modulates a cellular response mediated by a Glutamate Transporter Associated Protein. A method is further provided for identifying a compound that inhibits an interaction between a Glutamate Transporter Associated Protein and a glutamate transporter protein. A method is provided for treating a disorder associated with glutamate transport.

09/955,956 - 09/12/2001

FIGURE 1A

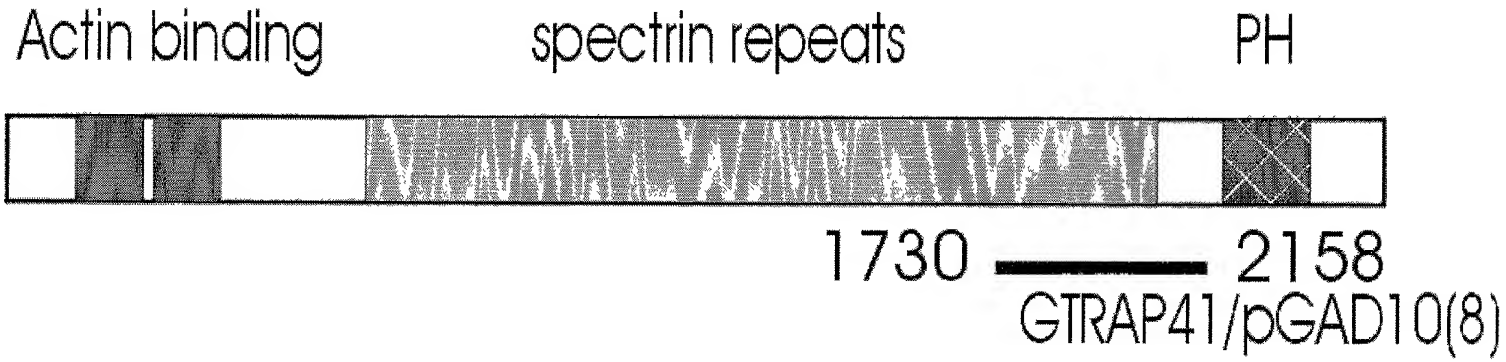

FIGURE 1B

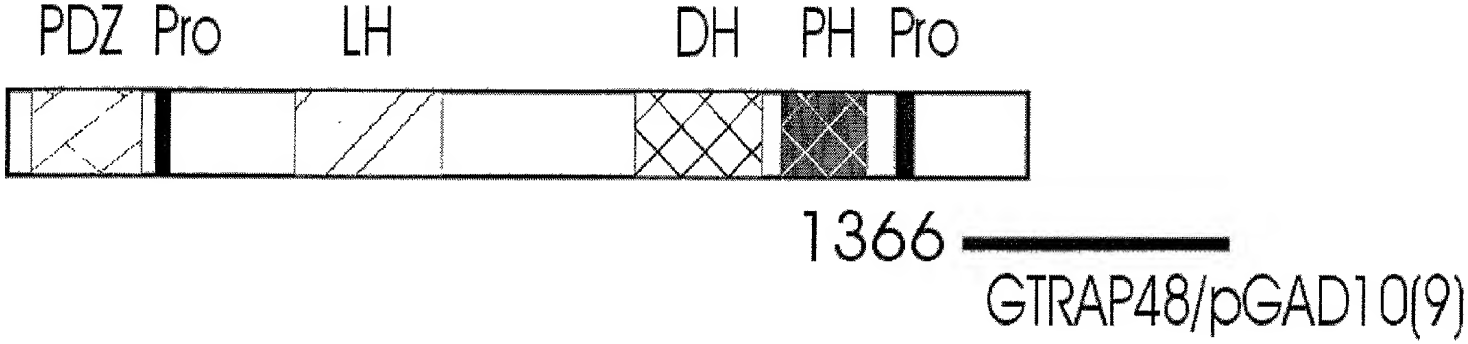

FIGURE 2

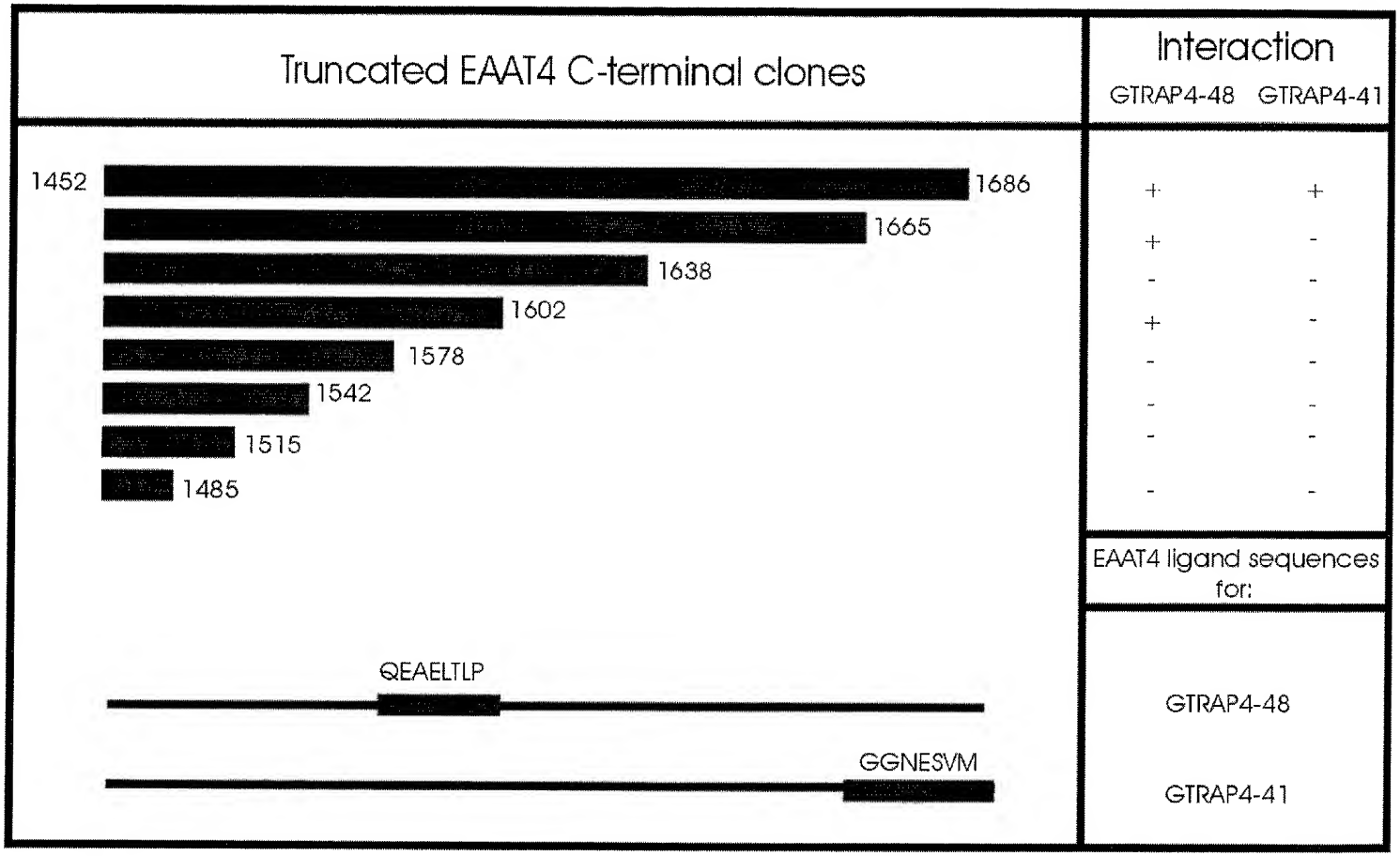
Truncated EAAT4 C-terminal clones
Interaction
GTRAP4-48 GTRAP4-41
1452
1686
1665
1638
1602
1578
1542
1515
1485
+ +
+ -
- -
+ -
- -
- -
- -
- -
EAAT4 ligand sequences for:
QEAELTLP
GGNESVM
GTRAP4-48
GTRAP4-41

FIGURE 3A

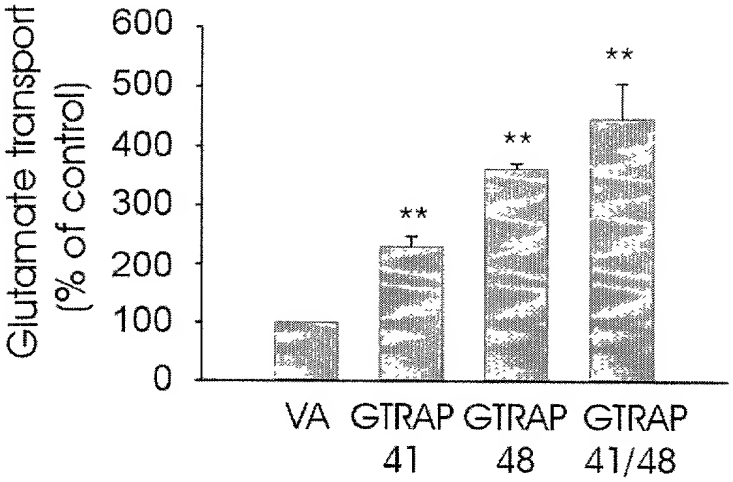

FIGURE 3B

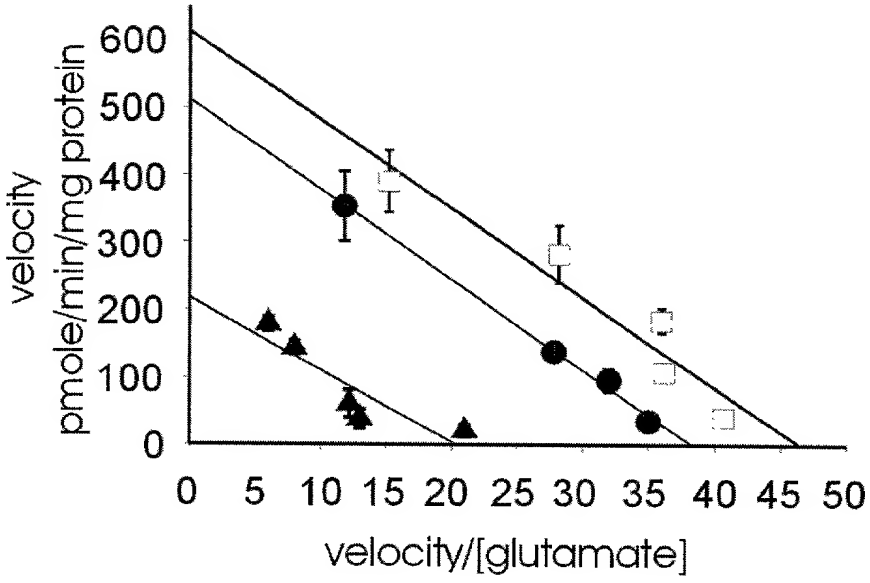

FIGURE 4A

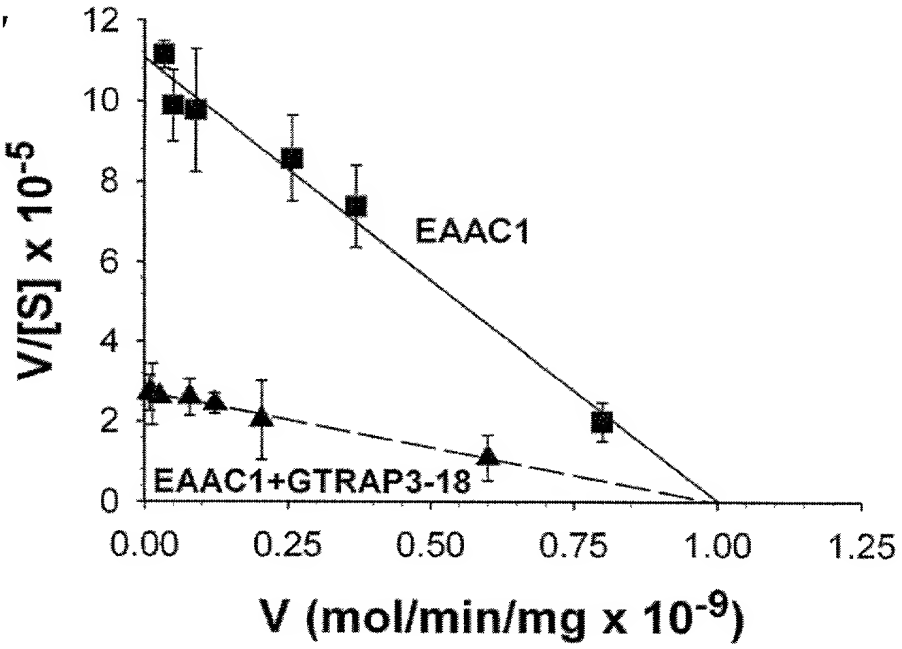

FIGURE 4B

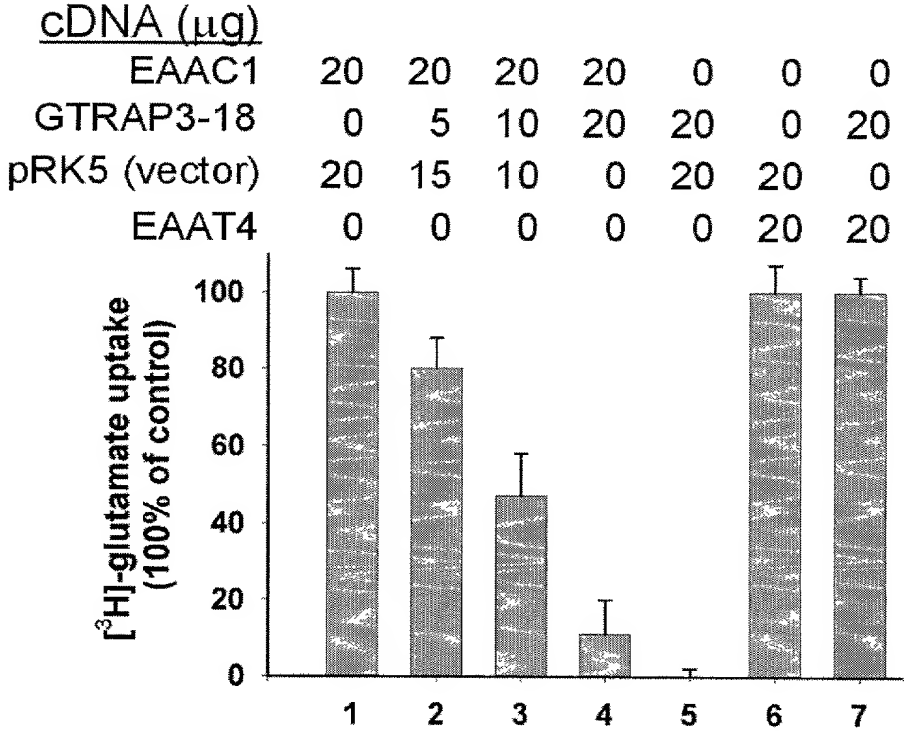

FIGURE 5A

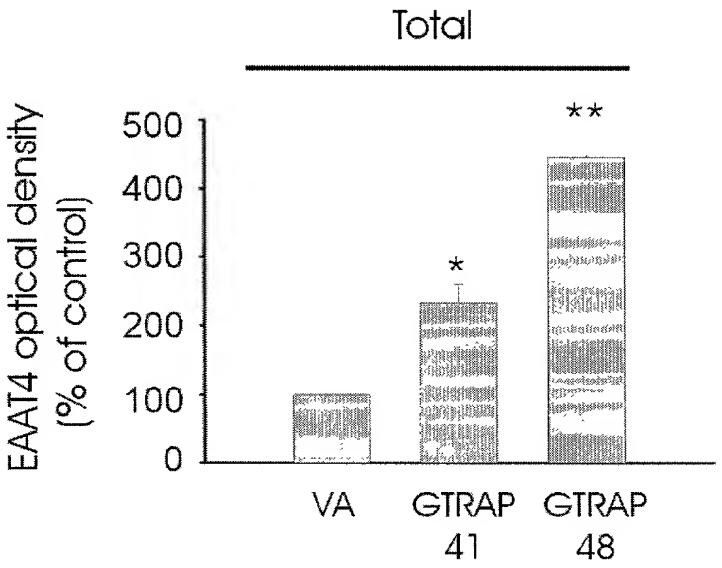

FIGURE 5B

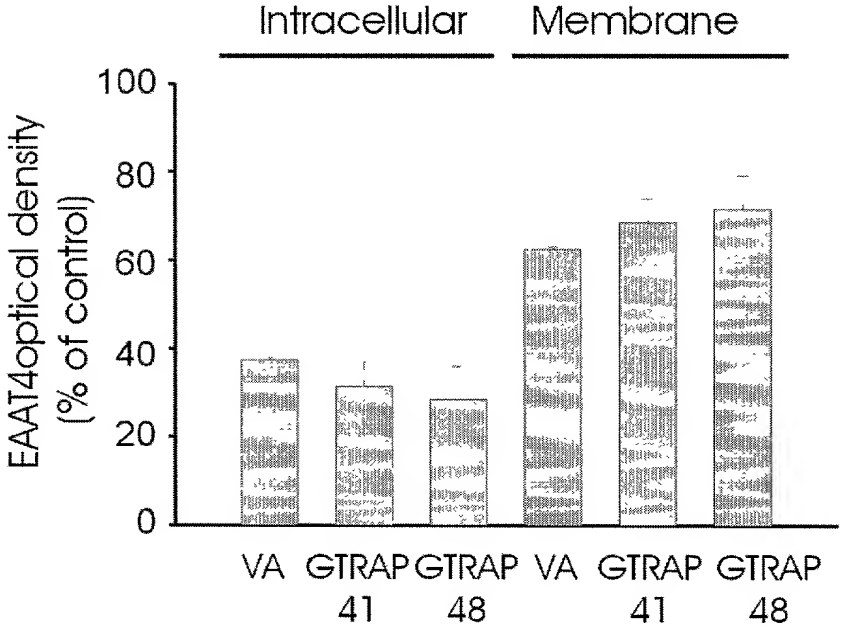

FIGURE 6A

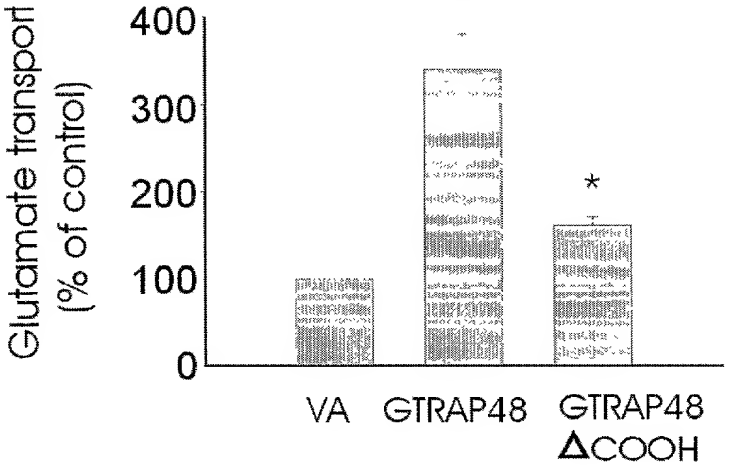

FIGURE 6B

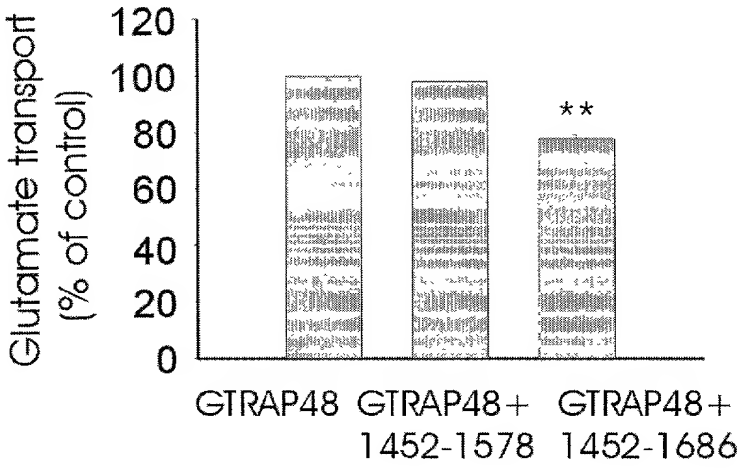

FIGURE 6C

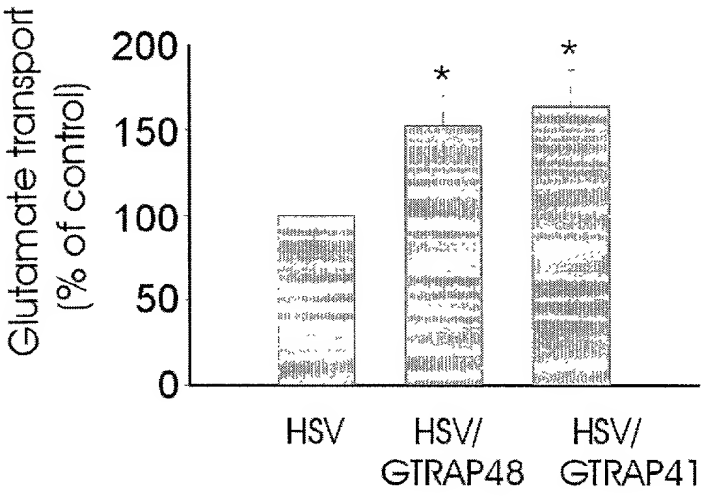

FIGURE 7A

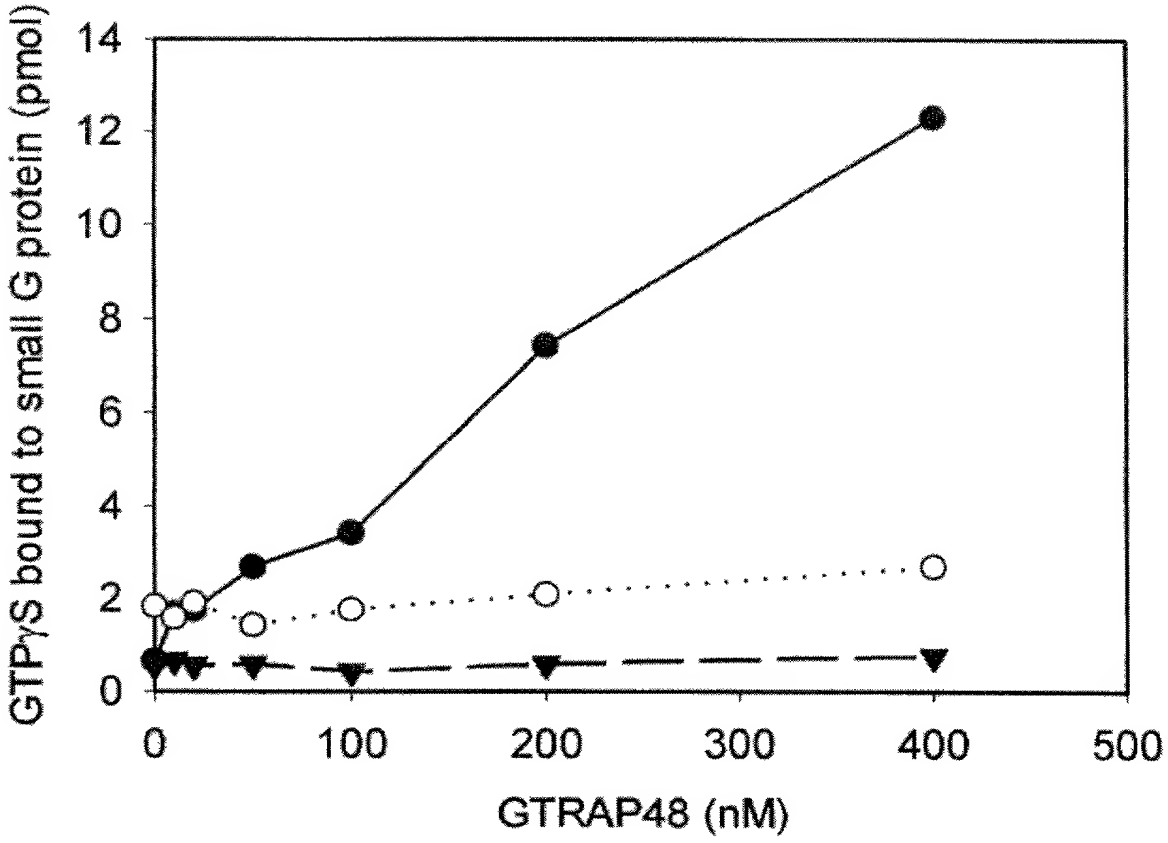

FIGURE 7B

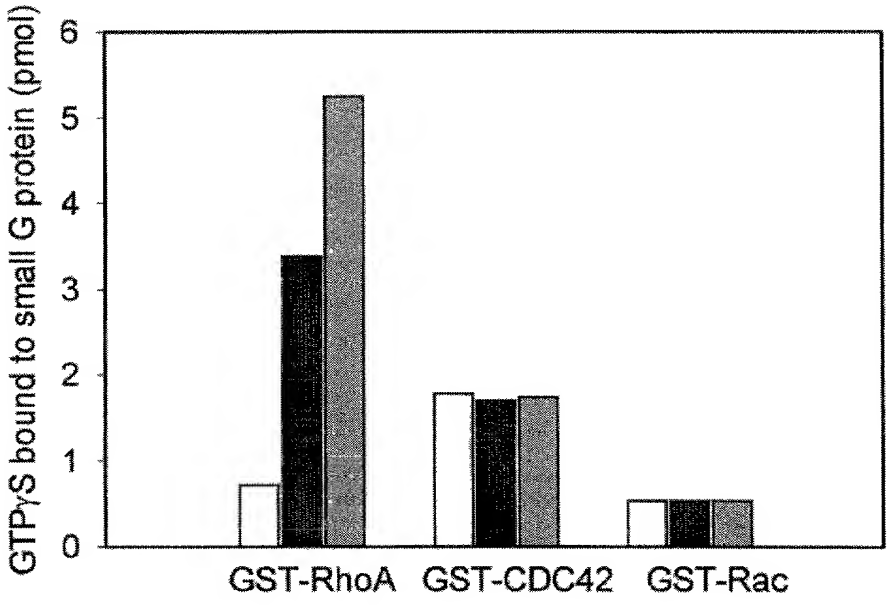

FIGURE 8A

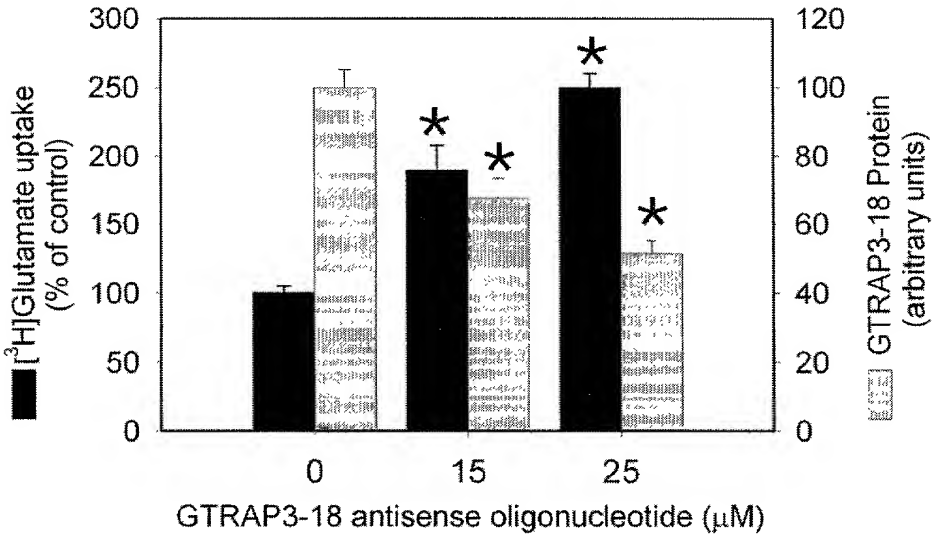

FIGURE 8B

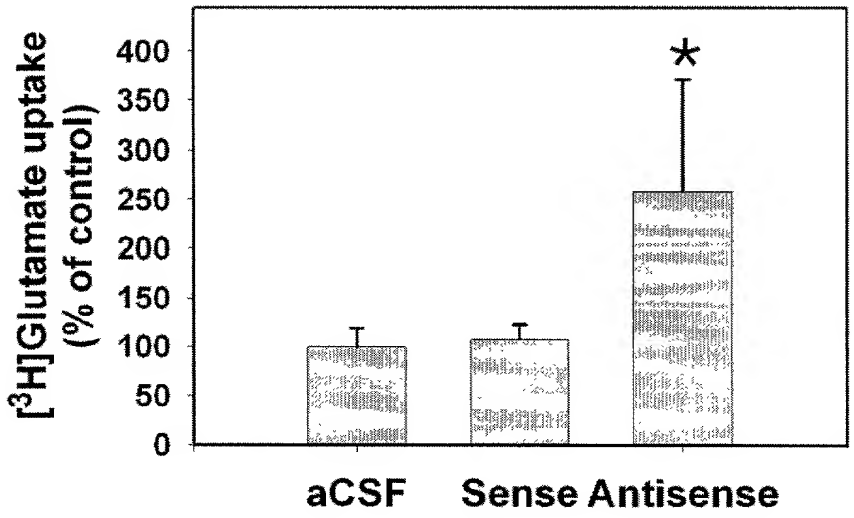

FIGURE 8C

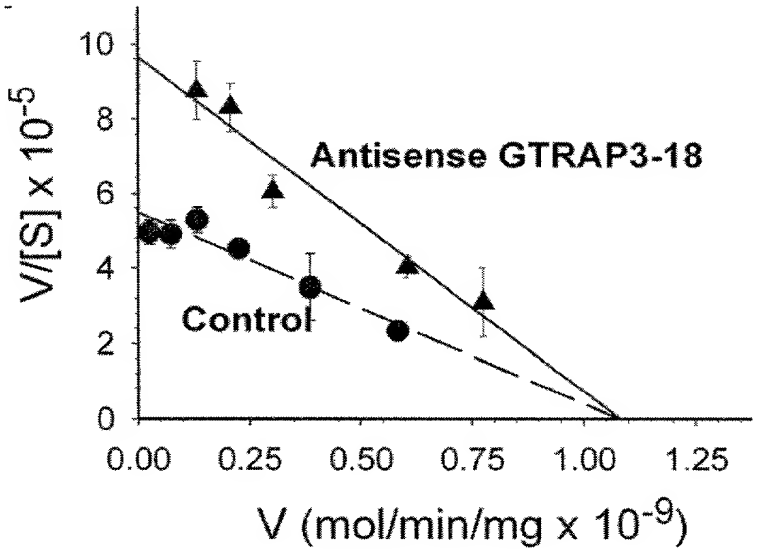

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FIGURE 9A

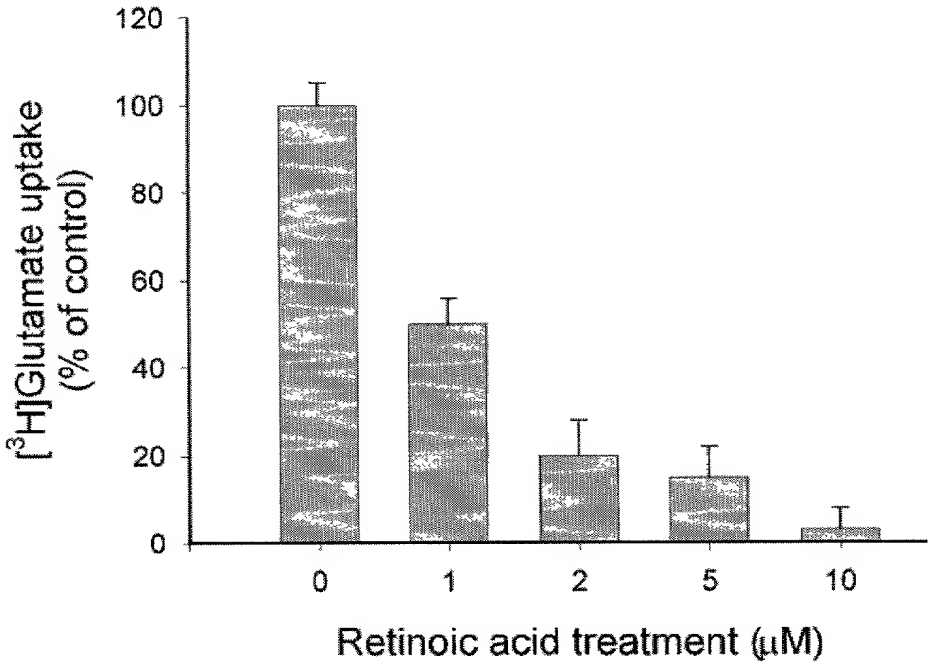


FIGURE 9B

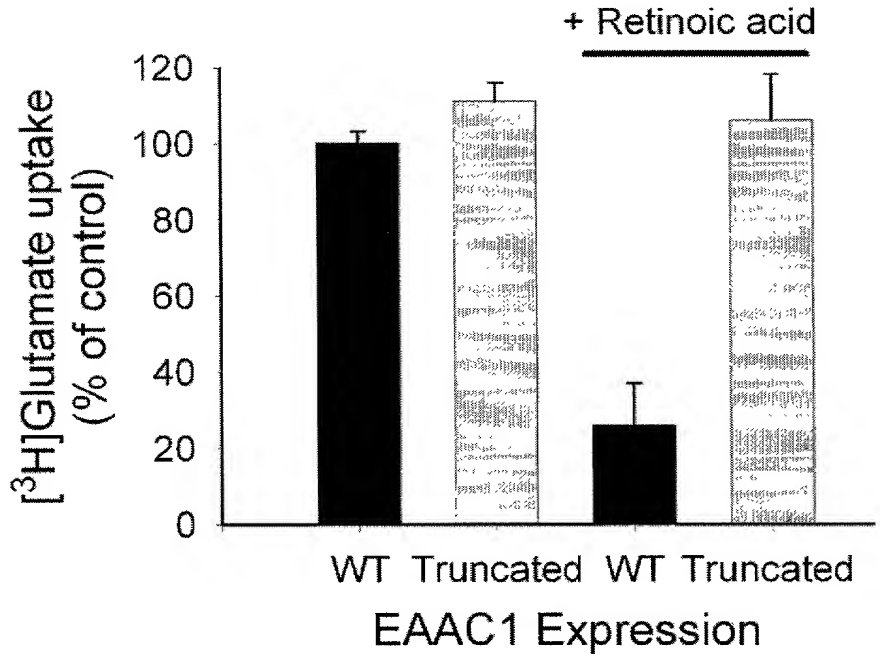


FIGURE 9C

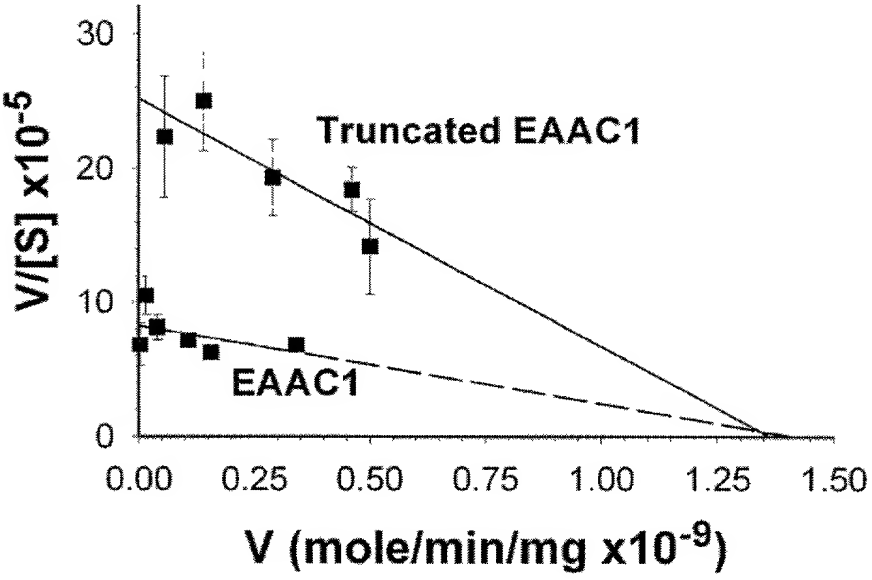


FIGURE 9D

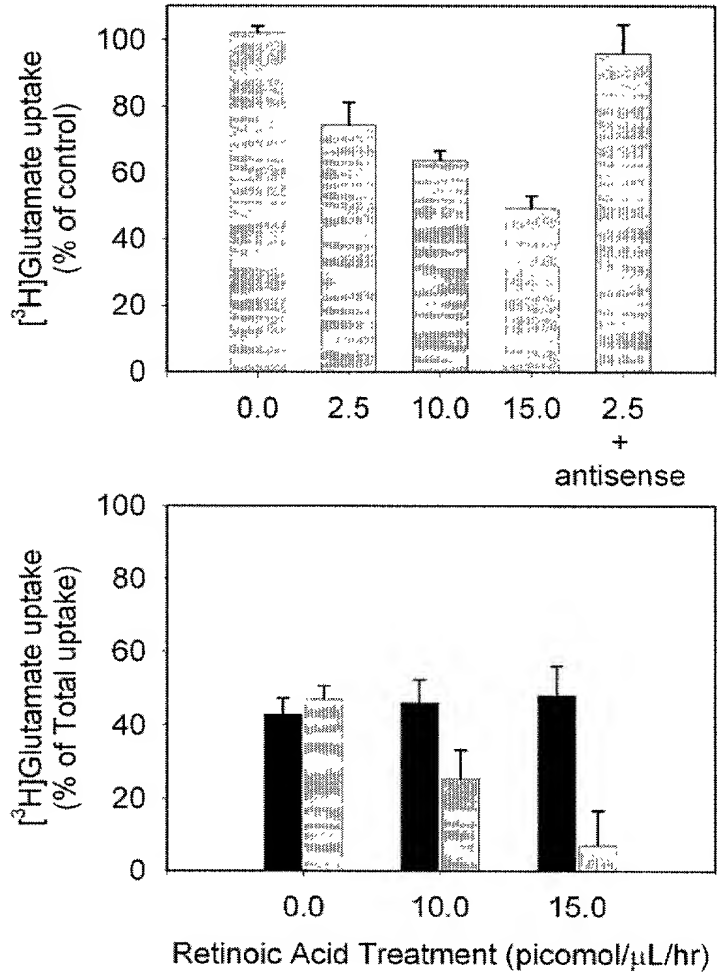
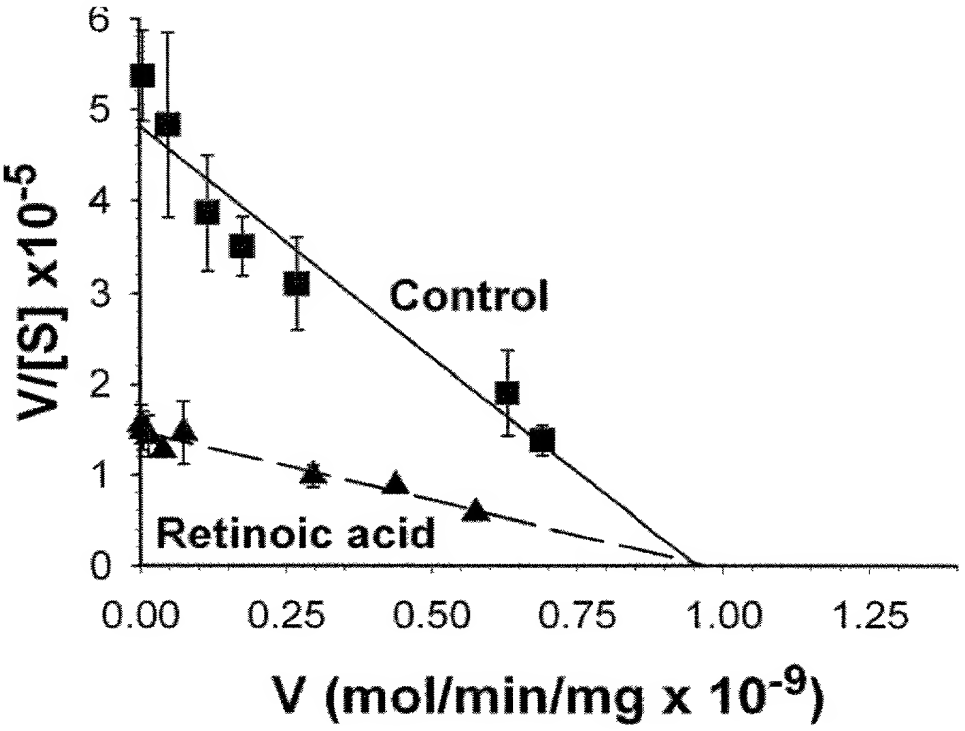


FIGURE 9E


6
5
4
3
2
1
0
V/[S] x10-5
Control
Retinoic acid
0.00
0.25
0.50
0.75
1.00
1.25
V (mol/min/mg x 10-9)

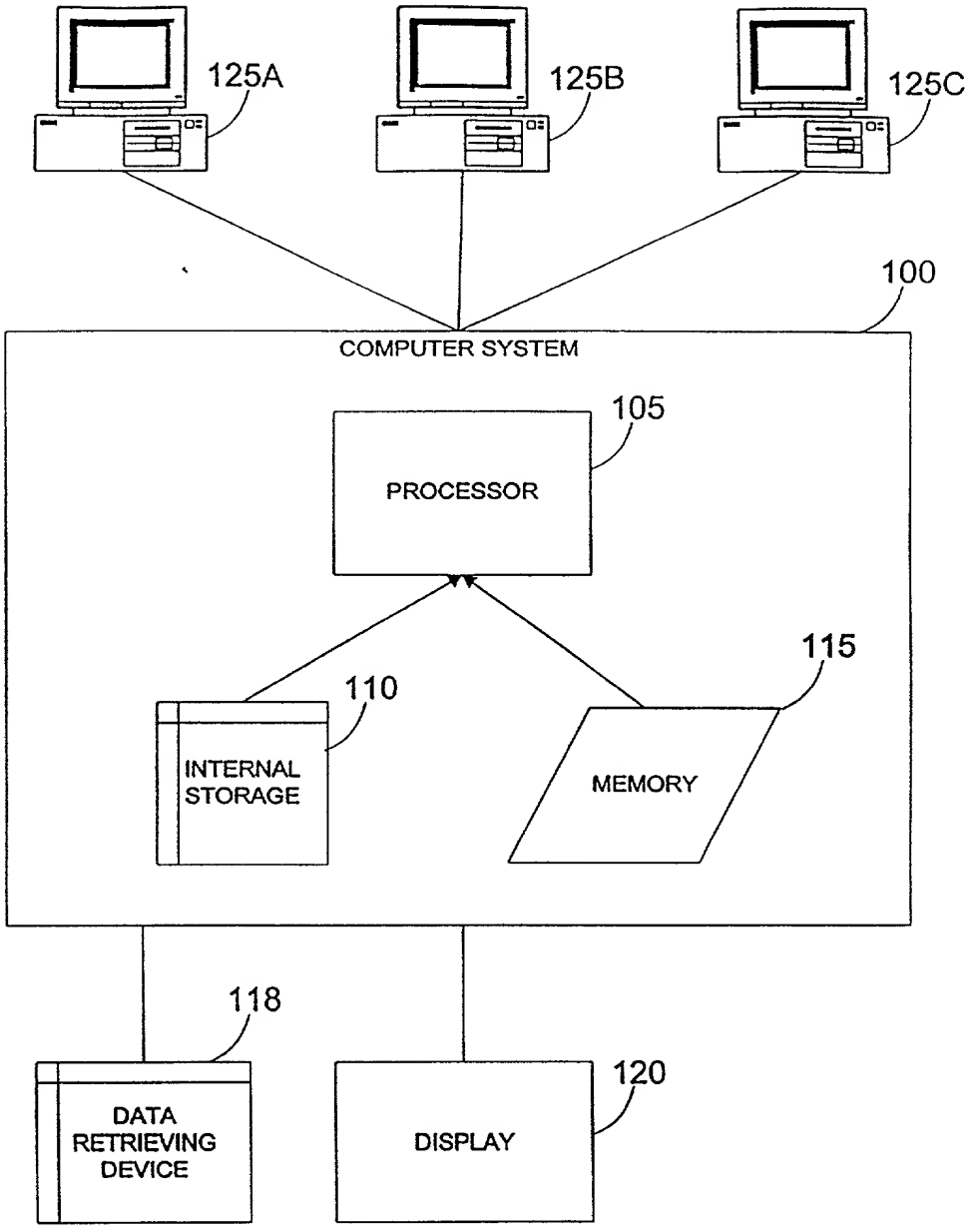

125A
125B
125C
100
COMPUTER SYSTEM
105
PROCESSOR
115
110
INTERNAL STORAGE
MEMORY
118
DATA RETRIEVING DEVICE
120
DISPLAY

FIGURE 10

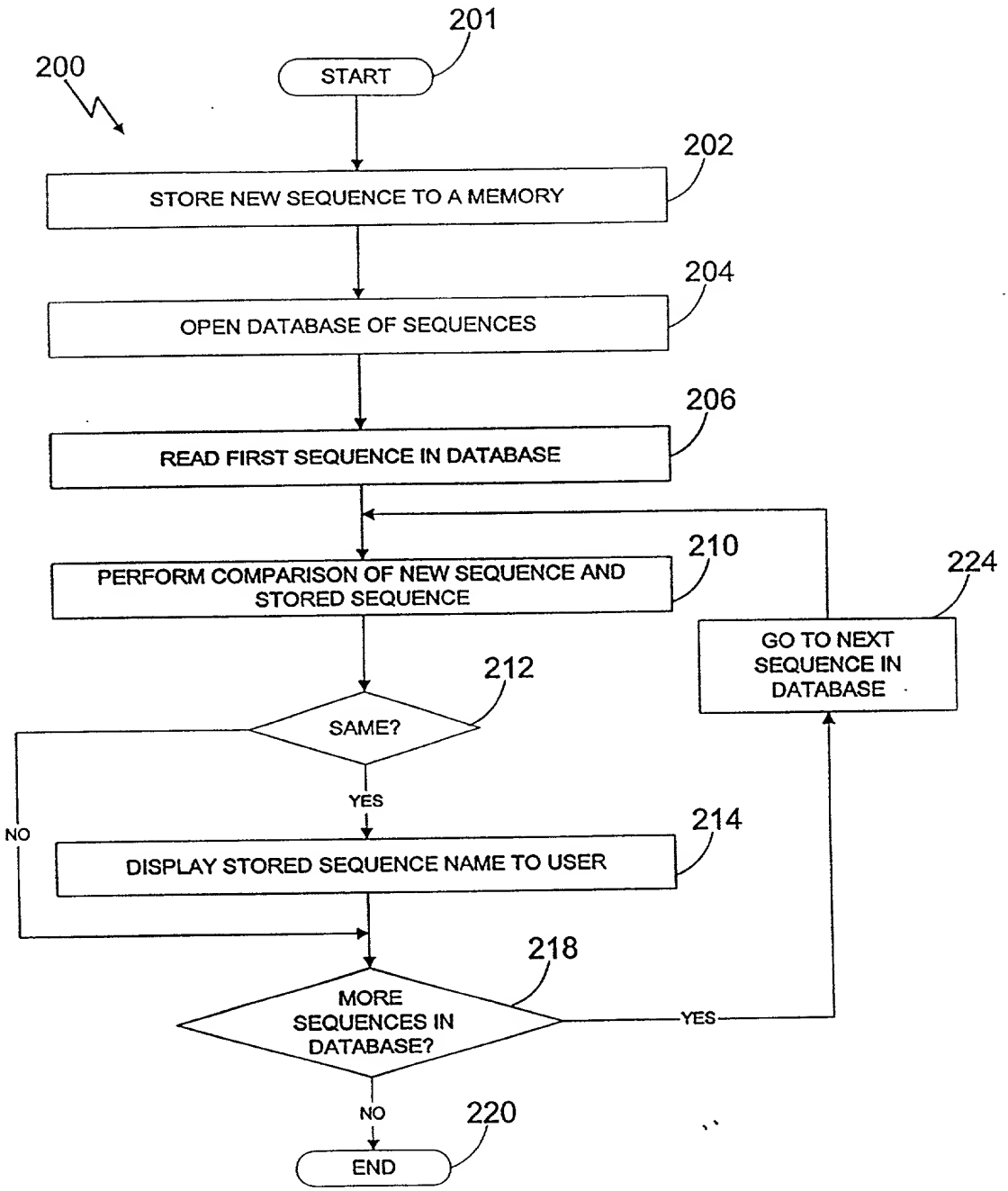

200
201
START
202
STORE NEW SEQUENCE TO A MEMORY
204
OPEN DATABASE OF SEQUENCES
206
READ FIRST SEQUENCE IN DATABASE
210
PERFORM COMPARISON OF NEW SEQUENCE AND STORED SEQUENCE
212
SAME?
NO
YES
214
DISPLAY STORED SEQUENCE NAME TO USER
218
MORE SEQUENCES IN DATABASE?
YES
NO
220
END
224
GO TO NEXT SEQUENCE IN DATABASE

FIGURE 11

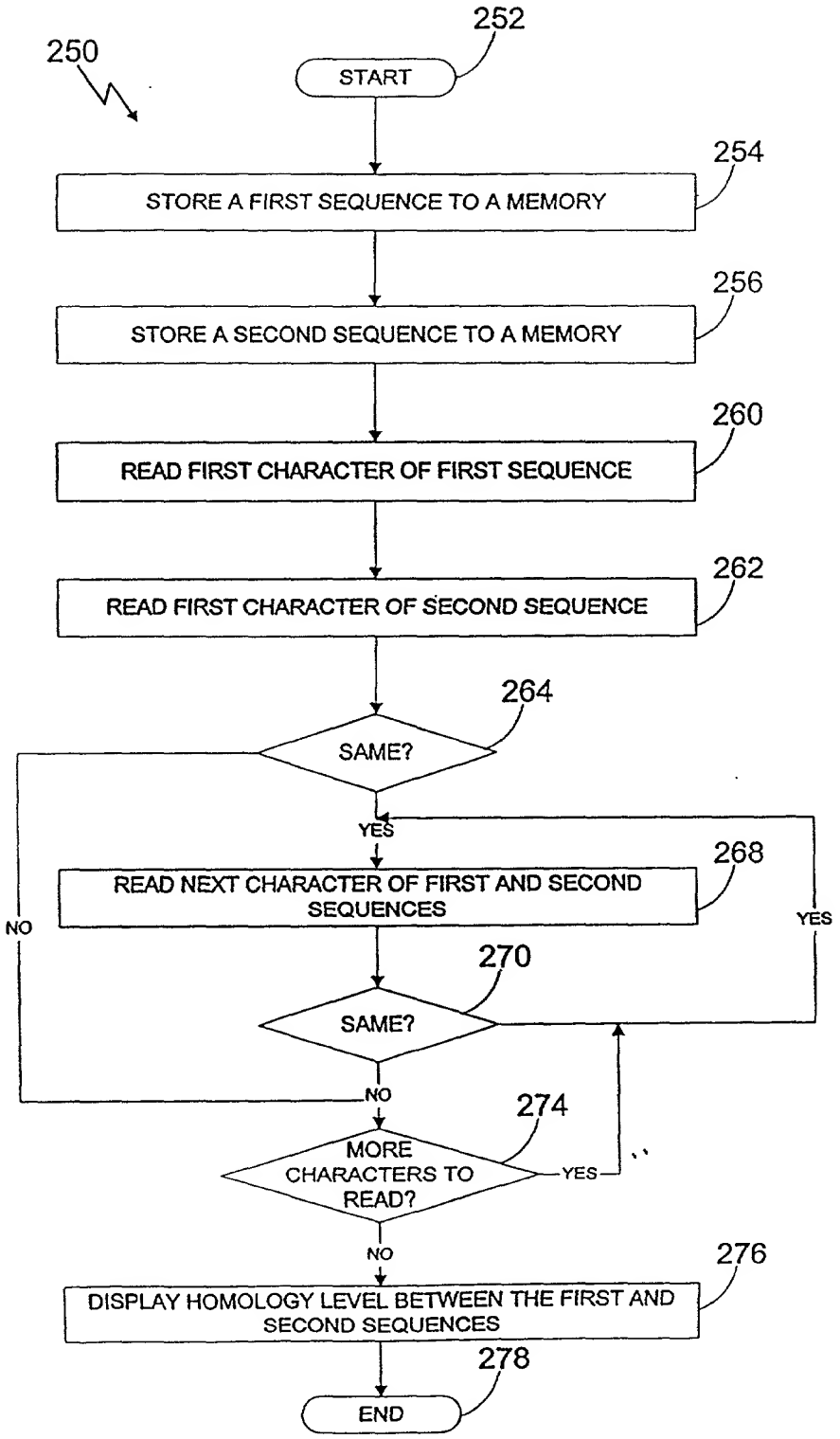

250
252
START
254
STORE A FIRST SEQUENCE TO A MEMORY
256
STORE A SECOND SEQUENCE TO A MEMORY
260
READ FIRST CHARACTER OF FIRST SEQUENCE
262
READ FIRST CHARACTER OF SECOND SEQUENCE
264
SAME?
YES
NO
268
READ NEXT CHARACTER OF FIRST AND SECOND SEQUENCES
YES
270
SAME?
NO
274
MORE CHARACTERS TO READ?
YES
NO
276
DISPLAY HOMOLOGY LEVEL BETWEEN THE FIRST AND SECOND SEQUENCES
278
END

FIGURE 12

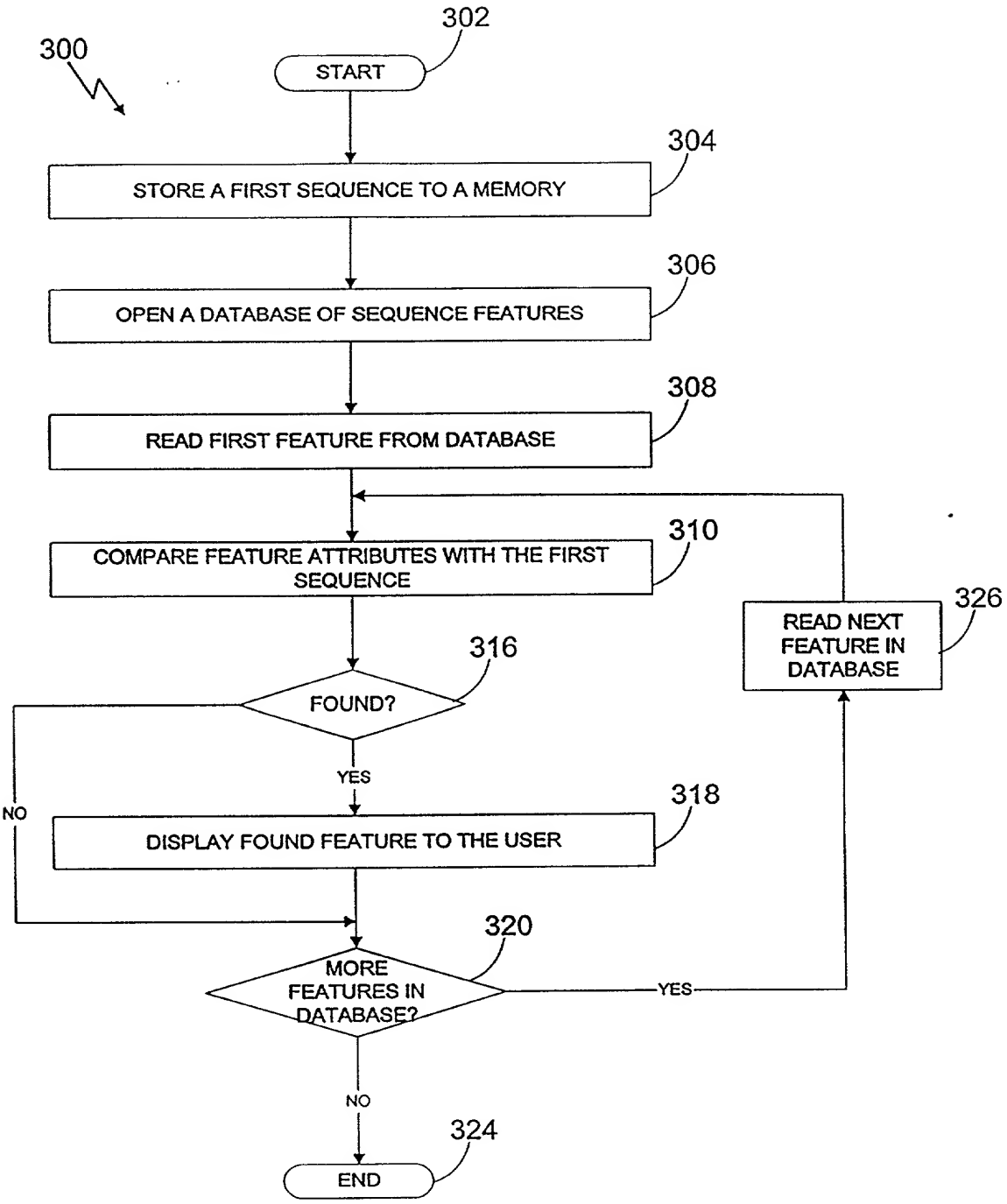

300
302
START
304
STORE A FIRST SEQUENCE TO A MEMORY
306
OPEN A DATABASE OF SEQUENCE FEATURES
308
READ FIRST FEATURE FROM DATABASE
310
COMPARE FEATURE ATTRIBUTES WITH THE FIRST SEQUENCE
316
FOUND?
YES
NO
318
DISPLAY FOUND FEATURE TO THE USER
320
MORE FEATURES IN DATABASE?
YES
NO
324
END
326
READ NEXT FEATURE IN DATABASE

FIGURE 13

FIGURE 14A

ATGAGCAGCACCCTGTCACCCACTGACTTCGACAGCTTGGAGATCCAGGGCCAGTACAGTGA
CATCAACAACCGCTGGGACCTGCCCGACTCAGATTGGGACAATGACAGCAGTTCAGCCCGCC
TCTTTGAGAGGTCCAGAATTAAGGCCCTGGCAGATGAGCGAGAAGCCGTGCAGAAGAAAACC
TTCACCAAGTGGGTGAACTCCCACCTGGCCCGGGTGACATGCCGGGTGGGAGACCTGTACAG
CGACCTGCGGGACGGGCGCAACCTCCTGAGGCTCCTGGAGGTGCTCTCGGGAGAGACCCTGC
CAAAACCCACCAAGGGCCGGATGCGGATTCACTGCCTGGAGAATGTCGACAAAGCACTGCAG
TTCCTGAAGGAGCAGAAGGTGCACCTGGAAAACATGGGCTCCCACGACATTGTGGATGGGAA
CCACCGTCTGACCCTTGGGCTAGTGTGGACCATCATCCTCCGATTTCAGATCCAAGACATCA
GTGTGGAGACAGAAGACAACAAGGAGAAGAAGTCAGCCAAGGATGCCCTGCTGCTGTGGTGC
CAGATGAAGACTGCAGGGTATCCCAATGTCAATGTGCACAACTTTACCACCAGTTGGAGAGA
TGGGCTGGCCTTTAATGCCATTGTGCACAAACACCGGCCAGACCTGTTGGATTTTGAGTCCC
TGAAGAAGTGTAACGCACACTACAATCTGCAGAATGCTTTCAATCTGGCTGAAAAGGAACTT
GGCCTGACGAAGCTCCTGGATCCTGAAGATGTGAACGTAGACCAACCCGATGAGAAGTCCAT
CATCACCTACGTGGCCACTTACTACCACTACTTCTCGAAGATGAAGGCCCTGGCTGTGGAAG
GCAAAAGGATTGGCAAGGTCCTGGACCATGCCATGGAGGCAGAACACCTGGTAGAGAAATAT
GAGTCCCTGGCCTCTGAACTGCTGCAGTGGATCGAGCAAACGATTGGGACCTTCAATGACCG
ACAGCTGGCCAACTCCCTGAGTGGCGTCCAGAACCAGCTGCAGTCTTTCAATTCCTACCGCA
CGGTGGAGAAGCCACCCAAGTTCACAGAGAAAGGGAACTTGGAGGTGTTGCTCTTCACCATC
CAGAGTAAGCTGCGGGCCAACAACCAGAAAGTCTACACACCACGCGAAGGCCGGCTCATCTC
GGACATCAACAAGGCCTGGGAGCGGCTAGAGAAAGCCGAACATGAGCGAGAGCTGGCCCTGC
GCACGGAGCTGATCCGCCAGGAGAAGCTGGAGCAACTGGCTGCTCGCTTCGACCGCAAGGCT
GCCATGCGGGAGACCTGGCTCAGTGAGAACCAGCGCCTCGTCTCCCAGGACAACTTTGGCCT
GGAGCTGGCAGCAGTGGAGGCAGCAGTGCGGAAGCATGAAGCCATTGAGACAGACATTGTGG
CCTACAGCGGCCGGGTGCAAGCGGTGGACGCCGTAGCCGCAGAACTGGCCGCTGAGCATTAC
CATGACATTAAGCGCATTGCGGCGCGGCAGAACAACGTGGCCCGGCTCTGGGACTTCTTACG
AGAGATGGTGGCCGCCCGCCGTGAGCGGCTCCTTCTCAACCTGGAGCTGCAGAAGGTGTTTC
AGGACCTGCTCTACCTCATGGACTGGATGGCAGAGATGAAGGGCCGGCTGCAGTCTCAGGAC
CTAGGCAAGCATCTGGCTGGAGTGGAAGATCTACTGCAACTACACGAACTGGTGGAGGCGGA
CATTGCAGTTCAGGCTGAGAGGGTGCGAGCGGTCAGCGCCTCTGCCCTGCGCTTCTGCGACC
CAGGGAAAGAGTATAGACCTTGCGGCCCGCAGCTAGTGTCAGAGAGGGTAGCCACTCTGGAG
CAGAGCTATGAGGCCCTGTGCGAATTGGCAGCAACTCGAAGGGCCCGACTGGAAGAGTCCCG
TCGTCTCTGGAGGTTCCTCTGGGAAGTGGGTGAGGCCGAGGCCTGGGTTCGGGAGCAGCAGC
ACCTCCTGGCCTCAGCTGAGACAGGCCGGGACCTGACTGGTGTCCTCCGCCTGCTCAATAAG
CACACAGCCCTACGGGGTGAGATGAGTGGCCGCCTGGGGCCCCTGAAGCTCACCCTGGAACA
AGGTCAGCAGTTAGTTGCCGAGGGCCACCCTGGAGCTAACCAAGCCTCAACCCGTGCAGCAG
AGCTCCAGGCCCAGTGGGAGCGACTAGAAGCCCTGGCCGAGGAGCGAGCCCAGCGGCTAGCA
CAGGCTGCCAGCCTCTACCAGTTCCAGGCAGATGCCAATGACATGGAGGCTTGGTTGGTGGA
CGCACTACGCCTGGTATCTAGCCCTGAGGTAGGGCACGATGAGTTCTCCACGCAGGCCCTGG
CCAGGCAGCACAGGGCCCTTGAGGAGGAGATCCGAGCCCACCGGCCTACACTGGATGCCTTG
AGGGAGCAGGCTGCAGCCCTGCCACCTGCACTGAGCCACACACCTGAGGTACAGGGCAGGGT
GCCCACTCTGGAGCAGCACTATGAGGAGCTGCAGGCCCGGGCAGGTGAGCGTGCACGAGCCC
TGGAAGCAGCCCTGGCGTTCTATACCATGCTCAGCGAGGCCGGGGCTTGTGGGCTCTGGGTA
GAGGAGAAGGAGCAGTGGCTCAACGGGCTGGCCCTACCTGAGCGCCTGGAGGACCCGGAGGT
GGTCCAACAGAGGTTTGAGACCTTAGAGCCCGAAATGAACGCCCTGGCTGCACGGATTACTG
CTGTCAGTGACATAGCTGAGCAGTTGCTGAAGGCCAGTCCACCAGGCAAGGACCGCATCATT
GGCACCCAGGAGCAGCTCAACCAAAGGTGGCAGCAGTTCAGGTCCCTGGCAGGTGGCAAAAA
GGCAGCTCTGACATCAGCCCTGAGCATCCAGAATTACCACCTAGAGTGCACAGAGACCCAGG
CCTGGATGAGAGAAAAGACCAAGGTCATTGAGTCTACCCAGGACCTAGGCAATGATCTAGCT

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FIGURE 14B

GGTGTGCTGGCCCTGCAGCGGAAGCTGGCAGGCACTGAGAGAGATCTGGAAGCCATCTCTGC
CCGGGTGGGTGAGCTGACCCAAGAGGCAAATGCTTTGGCTGCTGGGCACCCAGCCCAAGCCC
CTGCCATCAACACACGGCTTGGAGAGGTTCAAACTGGATGGGAGGATCTTCGGGCAACCATG
AGGCGGAGAGAAGAGTCCCTGGGTGAGGCTCGACGGCTGCAAGATTTCCTGCGCAGCTTAGA
TGACTTCCAGGCCTGGCTAGGCCGCACACAGACTGCTGTAGCCTCTGAGGAAGGACCAGCCA
CCCTTCCAGAGGCAGAAGCCCTCTTAGCCCAGCATGCAGCTCTGCGGGGAGAGGTGGAGAGA
GCCCAGAGCGAGTACAGCCGCCTCAGGACCTTGGGCGAGGAGGTGACCAGAGACCAGGCTGA
TCCCCAATGCCTCTTCCTCAGACAGAGGCTGGAAGCCCTTGGAACCGGCTGGGAGGAGCTGG
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AGAGATGCTCGCCAGGCTGAGGGAGTTCTCAGCAGCCAGGAATATGTTCTGTCTCACACGGA
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AAGGGCAATATCCATGCTGAGAAGATCCAAGAGAAGGCAGACTCCATCGAGAAGAGGCACAG
AAAGAACCAGGAGGCCGTGCAGCAGCTTCTAGGCCGCCTTCGGGACAACCGAGAGCAGCAGC
ACTTCTTGCAAGACTGTCAGGAGCTGAAACTCTGGATTGACGAGAAGATGCTGACAGCTCAG
GATGTGTCCTATGATGAGGCACGCAACCTGCACACCAAGTGGCAAAAACACCAGGCATTCAT
GGCCGAGCTGGCAGCCAACAAGGACTGGCTGGACAAAGTGGACAAGGAAGGGCGGGAGCTGA
CTCTTGAAAAGCCAGAACTCAAAGTCCTAGTGTCAGAGAAGCTGGAGGACCTGCACAGGCGC
TGGGATGAACTGGAGACTACCACCCAAGCCAAGGCCCGCAGTCTTTTTGATGCTAACCGGGC
AGAGCTATTTGCCCAAAGCTGTTCTGCCCTGGAGAGCTGGCTGGAGAGCCTGCAGGCCCAGC
TGCACTCAGATGACTATGGCAAGGACCTCACCAGTGTCAACATTCTGCTAAAGAAGCAACAG
ATGCTGGAACGAGAGATGGCTGTGAGAGAGAAGGAGGTAGAGGCTATCCAGGCCCAGGCAAA
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AGAAGTTCAGGGCCTTGTGTCAGCCCATGAAGGACCGCTGCCGGCGCCTGCAAGCCTCCCGA
GAGCAGCACCAGTTCCACCGGGATGTGGAGGATGAGATACTGTGGGTGACCGAGCGGCTTCC
CATGGCCAGCTCTCTGGAGCATGGCAAGGACTTGCCCAGCGTCCAGCTTCTCATGAAGAAAA
ACCAGACTCTGCAGAAGGAGATCCAGGGCCATGAGCCCCGGATTGCAGACCTCAAAGAGAGG
CAGCGCACTCTGAGAACAGCAGCAGCGGGTCCAGAGCTGGCTGAGCTCCAGGAAATGTGGAA
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GGCCAGGAAAAGGCCAAGGATGAGCTGAGCGCCCAGGCAGAAGTGAAGAAGCATCAGGTACT
AGAACAAGCCCTTGCTGACTATGCCCAGACCATCAAACAACTAGCAGCCAGCAGTCAAGATA
TGATTGACCATGAACATCCAGAGAGCACAAGGTTAACAATACGCCAAGCCCAGGTGGACAAG
CTGTACGCCGGCCTAAAGGAGCTGGCAGGAGAGCGGCGTGAGCGTCTGCAGGAGCACCTCAG
GCTGTGCCAGCTCCGCAGAGAGCTGGATGACCTGGAGCAGTGGATACAGGAGCGAGAAGTCG
TGGCAGCCTCCCATGAACTGGGCCAGGACTATGAGCATGTGACTATGCTTCGGGACAAATTC
CGAGAGTTCTCCAGGGACACCAGCACCATTGGCCAAGAGCGTGTAGACAGTGCCAATGCCCT
GGCCAATGGGCTCATTGCTGGGGGCCATGCTGCATGGGCCACCGTGGCCGAGTGGAAGGACA
GTCTCAATGAGGCCTGGGCTGACCTGCTGGAGCTGCTGGACACAAGAGGTCAGGTGCTGGCT
GCTGCTTATGAGCTGCAGCGCTTCCTGCATGGGGCCCGCCAAGCCCTGGCACGGGTGCAGCA
CAAGCAGCAGCAGCTTCCAGATGGGACGGGCCGCGACCTCAATGCTGCTGAGGCCCTGCAGC
GCCGGCACTGCGCCTATGAGCACGACATCCAAGCCCTCAGCACTCAGGTCCAGCAGGTTCAG
GACGATGGCCTCAGGCTACAAAAGGCCTATGCTGGAGACAAGGCTGAGGAAATTGGCCGTCA
CATGCAGGCAGTGGCTGAGGCGTGGGCCCAGCTCCAGGGAAGTTCTGCTGCCCGTCGCCAGC
TGTTACTGGATACCACAGACAAATTCCGATTCTTCAAGGCTGTCCGGGAGTTGATGCTGTGG
ATGGATGGGATTAACCTGCAGATGGATGCCCAGGAGAGGCCCCGGGATGTGTCCTCTGCAGA
TTTAGTCATCAAAAACCAACAAGGCATCAAAGCAGAGATAGAGGCAAGAGCTGACAGGTTCT
CCGCCTGCATTGACATGGGGCAAGAGCTGCTGGCCCGGAACCACTATGCCGCTGAGGAGATC

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FIGURE 14C

TCAGAGAAGCTGTCTCAGCTACAGTCCCGGCGCCAGGAGACAGCTGAAAAGTGGCAGGAGAA
GATGGACTGGCTACAGCTTGTTTTGGAGGTGCTTGTGTTTGGGAGAGATGCAGGCATGGCAG
AGGCCTGGCTATGCAGTCAGGAGCCATTGGTGCGAAGTGCAGAACTGGGTTGCACTGTGGAT
GAAGTAGAGAGCCTCATCAAGCGGCATGAAGCCTTCCAGAAGTCAGCAGTGGCCTGGGAGGA
GCGTTTCAGTGCCCTGGAGAAGCTCACTGCGCTGGAAGAGCGGGAGAATGAGCAGAAAAGGA
AGAGGGAGGAGGAGGAACGAAGGAAACAGCCCCCTACTTCAGAGCCCATGGCTAGTCAACCG
GAAGGGAGTCTGGTAGATGGCCAGAGAGTTCTTGACACTGCCTGGGATGGGACCCAGTCAAA
ATTGCCACCATCCACACAAGCACCCAGCATTAATGGGGTCTGCACGGACACGGAGTCCTCAC
AGCCTCTGTTGGAACAGCAAAGACTTGAACAGAGCAATGTCCCAGAAGGGCCTGGATCTGGC
ACAGGAGACGAGTCCAGCGGGCCCCGGGGAGAGAGGCAGACCCTGCCCCGGGGCCCTGCTCC
GTCTCCAATGCCCCAGAGCAGATCGTCTGAGTCAGCTCATGTTGCCACCCTGCCCGCACGAG
GTGCTGAGCTCTCTGCTCAGGAACAGATGGAAGGGACGCTGTGCCGCAAACAGGAGATGGAA
GCCTTCAATAAGAAAGCTGCCAACAGGTCCTGGCAGAATGTGTACTGTGTACTTCGGCGTGG
AAGCCTCGGCTTTTACAAGGATGCCAGGGCAGCTAGTGCAGGAGTGCCATACCATGGAGAAG
TGCCTGTCAGTCTGGCCAGGGCCCAGGGCAGTGTGGCCTTTGATTATCGGAAACGCAAACAT
GTCTTCAAGCTGGGCTTGCAGGATGGGAAAGAGTATCTATTCCAGGCCAAGGATGAGGCAGA
GATGAGCTCATGGCTGAGAGTGGTGAATGCAGCCATTGCCACTGCGTCCTCGGCCTCTGGAG
AGCCAGAAGAGCCAGTGGTGCCCAGTGCCAGCCGGGGTCTGACCAGGGCCATGACCATGCCC
CCAGTGTCACAGCCTGAGGGCTCCATCGTGCTTCGCAGCAAGGATGGCAGAGAAAGAGAGCG
AGAAAAACGATTCAGCTTCTTTAAGAAGAACAAGTAGTTGGGGCAAGACTCCCAGGCCAGCT
CCCTCCCTCTGTTCAGGAAACTGCCAGGGACTGTCGACAGAGACCACC

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FIGURE 15

MSSTLSPTDFDSLEIQGQYSDINNRWDLPDSDWDNDSSSARLFERSRIKALADEREA
VQKKTFTKWVNSHLARVTCRVGDLYSDLRDGRNLLRLLEVLSGETLPKPTKGRMRIHCLENV
DKALQFLKEQKVHLENMGSHDIVDGNHRLTLGLVWTIILRFQIQDISVETEDNKEKKSAKDA
LLLWCQMKTAGYPNVNVHNFTTSWRDGLAFNAIVHKHRPDLLDFESLKKCNAHYNLQNAFNL
AEKELGLTKLLDPEDVNVDQPDEKSIITYVATYYHYFSKMKALAVEGKRIGKVLDHAMEAEH
LVEKYESLASELLQWIEQTIGTFNDRQLANSLSGVQNQLQSFNSYRTVEKPPKFTEKGNLEV
LLFTIQSKLRANNQKVYTPREGRLISDINKAWERLEKAEHERELALRTELIRQEKLEQLAAR
FDRKAAMRETWLSENQRLVSQDNFGLELAAVEAAVRKHEAIETDIVAYSGRVQAVDAVAAEL
AAEHYHDIKRIAARQNNVARLWDFLREMVAARRERLLLNLELQKVFQDLLYLMDWMAEMKGR
LQSQDLGKHLAGVEDLLQLHELVEADIAVQAERVRAVSASALRFCDPGKEYRPCGPQLVSER
VATLEQSYEALCELAATRRARLEESRRLWRFLWEVGEAEAWVREQQHLLASAETGRDLTGVL
RLLNKHTALRGEMSGRLGPLKLTLEQGQQLVAEGHPGANQASTRAAELQAQWERLEALAEER
AQRLAQAASLYQFQADANDMEAWLVDALRLVSSPEVGHDEFSTQALARQHRALEEEIRAHRP
TLDALREQAAALPPALSHTPEVQGRVPTLEQHYEELQARAGERARALEAALAFYTMLSEAGA
CGLWVEEKEQWLNGLALPERLEDPEVVQQRFETLEPEMNALAARITAVSDIAEQLLKASPPG
KDRIIGTQEQLNQRWQQFRSLAGGKKAALTSALSIQNYHLECTETQAWMREKTKVIESTQDL
GNDLAGVLALQRKLAGTERDLEAISARVGELTQEANALAAGHPAQAPAINTRLGEVQTGWED
LRATMRRREESLGEARRLQDFLRSLDDFQAWLGRTQTAVASEEGPATLPEAEALLAQHAALR
GEVERAQSEYSRLRTLGEEVTRDQADPQCLFLRQRLEALGTGWEELGRMWESRQGRLAQAHG
FQGFLRDARQAEGVLSSQEYVLSHTEMPGTLQAADAAIKKLEDFMSTMDANGERIRGLLEAG
RQLVSKGNIHAEKIQEKADSIEKRHRKNQEAVQQLLGRLRDNREQQHFLQDCQELKLWIDEK
MLTAQDVSYDEARNLHTKWQKHQAFMAELAANKDWLDKVDKEGRELTLEKPELKVLVSEKLE
DLHRRWDELETTTQAKARSLFDANRAELFAQSCSALESWLESLQAQLHSDDYGKDLTSVNIL
LKKQQMLEREMAVREKEVEAIQAQAKALAQEDQSAGEVERTSRAVEEKFRALCQPMKDRCRR
LQASREQHQFHRDVEDEILWVTERLPMASSLEHGKDLPSVQLLMKKNQTLQKEIQGHEPRIA
DLKERQRTLRTAAAGPELAELQEMWKRLSHELELRGKRLEEALRAQQFYRDAAEAEAWMGEQ
ELHMMGQEKAKDELSAQAEVKKHQVLEQALADYAQTIKQLAASSQDMIDHEHPESTRLTIRQ
AQVDKLYAGLKELAGERRERLQEHLRLCQLRRELDDLEQWIQEREVVAASHELGQDYEHVTM
LRDKFREFSRDTSTIGQERVDSANALANGLIAGGHAAWATVAEWKDSLNEAWADLLELLDTR
GQVLAAAYELQRFLHGARQALARVQHKQQQLPDGTGRDLNAAEALQRRHCAYEHDIQALSTQ
VQQVQDDGLRLQKAYAGDKAEEIGRHMQAVAEAWAQLQGSSAARRQLLLDTTDKFRFFKAVR
ELMLWMDGINLQMDAQERPRDVSSADLVIKNQQGIKAEIEARADRFSACIDMGQELLARNHY
AAEEISEKLSQLQSRRQETAEKWQEKMDWLQLVLEVLVFGRDAGMAEAWLCSQEPLVRSAEL
GCTVDEVSLIKRHEAFQKSAVAWEERFSALEKLTALEERENEQKRKREEEERRKQPPTSEPM
ASQPEGSLVDGQRVLDTAWDGTQSKLPPSTQAPSINGVCTDTESSQPLLEQQRLEQSNVPEG
PGSGTGDESSGPRGERQTLPRGPAPSPMPQSRSSESAHVATLPARGAELSAQEQMEGTLCRK
QEMEAFNKKAANRSWQNVYCVLRRGSLGFYKDARAASAGVPYHGEVPVSLARAQGSVAFDYR
KRKHVFKLGLQDGKEYLFQAKDEAEMSSWLRVVNAAIATASSASGEPEEPVVPSASRGLTRA
MTMPPVSQPEGSIVLRSKDGRERERERKRFSFFKKNK.

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FIGURE 16A

ATGAGCATACGATTGCCCCATAGTATAGACAGATCAGCCAGTAAAAAGCAGTCTCAC
CTGTCCAGTCCCATTGCATCCTGGTTAAGTAGCCTGTCTTCTCTGGGAGATTCTACA
CCTGAACGCACATCCCCTTCTCACCACCGCCAGCCCTCTGACACTTCTGAGACAACA
GCAGGTCTTGTTCAGCGCTGTGTCATCATCCAAAAGGACCAGCATGGCTTTGGCTTC
ACAGTTAGTGGAGATCGCATTGTACTGGTGCAGTCCGTGCGCCCTGGAGGCGCAGCC
ATGAAAGCTGGTGTGAAAGAGGGTGACCGGATCATCAAAGTAAACGGCACCATGGTG
ACCAATAGCTCACACCTGGAGGTGGTAAAGCTTATCAAATCTGGCGCCTATGCTGCG
CTTACCCTCCTAGGCTCTTCTCCTCCCTCCGTCGGCGTCTCTGGGCTCCAGCAGAAT
CCATCTGTGGCAGGAGTGCTCAGAGTTAACCCCATCATTCCTCCACCACCTCCCCCG
CCACCCTTGCCACCTCCACAGCACATTACTGGACCCAAACCTCTTCAGGATCCTGAA
GTCCAAAAGCACGCCACTCAAATCCTCTGGAATATGCTAAGACAGGAGGAGGAAGAG
TTACAGGACATACTTCCACCCTGTGGTGAGACCAGTCAGAGAACATGTGAGGGCCGC
CTCTCTGTGGACTCCCAGGAGGCAGACAGTGGCTTGGATTCTGGGACAGAACGCTTT
CCCTCCATCAGTGAGTCATTGATGAATCGGAACTCAGTATTGTCAGATCCTGGACTA
GACAGCCCTCAAACCTCCCCTGTAATCCTGGCCAGGGTGGCCCAGCACCACAGGCGA
CAGGGCTCAGATGCAGCGTTGCTCCCGCTCAACCACCAGGGTATAGATCAAAGCCCA
AAGCCTCTGATTATTGGCCCAGAGGAAGATTATGACCCAGGTTATTTCAACAATGAG
AGTGACATCATCTTCCAAGATCTTGAAAAACTGAAGTCACATCCAGCTTACTTGGTA
GTTTTTCTACGTTACATCCTCTCTCAGGCAGACCCTGGCCCCCTGCTTTTTTATTTG
TGTTCAGAAGTTTATCAACAGACAAATCCCAAAGATTCCCGAAGTCTGGGGAAAGAC
ATCTGGAACATTTTCCTGGAGAAAAATGCGCCTCTCAGAGTGAAGATCCCTGAGATG
TTGCAGGCTGAAATTGACCTACGCCTGCGGAACAATGAGGACCCTCGCAATGTGCTC
TGTGAAGCTCAGGAGGCAGTCATGCTGGAAATCCAGGAGCAGATCAACGACTACAGA
TCCAAGCGTACTCTGGGCCTGGGCAGCCTCTATGGTGAAAATGACCTGCTAGGCCTG
GATGGGGACCCTCTTCGAGAACGCCAAATGGCTGAGAAGCAGCTGGCTGCCCTTGGA
GATATCTTGTCCAAATATGAGGAAGATCGGAGTGCCCCCATGGACTTTGCTGTTAAT
ACCTTTATGAGCCACGCTGGGATCCGTCTTCGGGAGTCTCGATCCTCCTGCACGGCA
GAAAAGACCCAGTCTGCCCCTGACAAGGACAAGTGGCTGCCCTTCTTCCCTAAGACC
AAGAAGCAGAGCAGCAATTCCAAGAAAGAAAAGGATGCCTTGGAGGACAAGAAGCGA
AACCCCATCCTCAGATATATTGGGAAGCCCAAGAGCTCCTCTCAGAGCATTAAGCCA
GGCAATGTGAGGAACATCATTCAGCACTTTGAGAACAGCCATCAGTATGATGTCCCA
GAGCCGGGGACACAACGACTCTCAACAGGAAGCTTTCCTGAGGACCTGCTGGAGAGT
GACAGTTCGCGCTCAGAGATTCGACTGGGCCGCTCTGGGAGCCTCAAGGGCCGGGAA
GAGATGAAGCGATCCCGGAAAGCAGAGAACGTGCCCCGGCCTCGAAGTGACGTTGAC
ATGGATGCTGCTGCAGAGGCTGCCCGCCTTCACCAGTCAGCCTCGTCCTCTGCCTCC
AGCCTCTCCACCAGGTCTCTTGAGAACCCAACCCCTCCCTTCACCCCCAAAATGGGC
CGCAGGAGCATTGAGTCCCCCAATCTGGGGTTCTGTACAGACGTCATCCTTCCCCAC
CTCCTGGAGGATGATCTGGGCCAATTGTCTGACCTGGAGCCAGAGCCAGAGGTCCAA
AACTGGCAGCATACAGTAGGCAAGGATGTGGTGGCCAACCTGACCCAGAGGGAAATT
GACCGGCAAGAGGTCATCAATGAGCTTTTTGTGACAGAAGCATCTCACCTGCGCACA
CTCCGAGTCCTGGACCTCATCTTCTACCAGCGCATGAGAAAGGAGAACCTAATGCCT
CGGGAAGAGCTAGCGCGGCTCTTCCCTAACCTGCCTGAGCTCATAGAGATTCACAAT
TCCTGGTGTGAGGCCATGAAGAAGCTCCGGGAGGAGGGCCCCATTATCAGAGACATC
AGTGACCCCATGCTGGCTCGGTTTGATGGTCCTGCCCGAGAAGAACTCCAGCAAGTA
GCTGCACAATTCTGTTCCTATCAGTCAGTAGCCCTAGAGCTAATCAGGACTAAGCAA
CGTAAGGAGAGTCGGTTCCAGCTCTTCATGCAGGAGGCTGAGAGCCACCCTCAGTGC
CGGCGTCTGCAGCTCCGAGACCTCATCGTCTCTGAAATGCAACGGCTCACCAAGTAC
CCACTGCTGCTAGAGAACATCATCAAGCACACAGAGGGTGGCACCTCTGAGCATGAG

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FIGURE 16B

AAGCTCTGCCGTGCCCGGGACCAGTGCCGGGAGATTCTCAAGTTTGTGAATGAAGCA
GTAAAGCAGACAGAGAACCGCCACCGGCTAGAGGGGTACCAGAAACGCCTGGATGCC
ACTGCCCTAGAGCGGGCCAGCAACCCCTTGGCAGCAGAGTTCAAGAGCCTGGATCTT
ACAACAAGGAAGATGATCCACGAGGGGCCTCTGACCTGGAGGATCAGCAAAGACAAG
ACCCTGGACCTCCAGGTGCTTCTGCTTGAGGACCTGGTGGTACTGCTGCAGAGACAA
GAGGAGCGGCTGCTGCTAAAGTGCCACAGCAAGACAGCCGTGGGCTCCTCCGACAGC
AAGCAGACGTTCAGCCCTGTGCTGAAGCTCAATGCTGTGCTCATCCGCTCCGTGGCT
ACAGACAAGCGAGCCTTCTTCATCATCTGCACCTCCGAGCTGGGCCCTCCCCAGATC
TATGAGCTGGTTGCATTGACGTCATCAGACAAGAATATATGGATGGAGCTCTTAGAA
GAGGCCGTGCAGAATGCCACCAAGCACCCTGGAGCTGCCCCAATCCCCATCCATCCC
TCACCACCAGGATCCCAGGAGCCGGCCTACCAGGGCTCCACCTCCAGCAGGGTAGAA
ATAAATGACTCAGAAGTATATCACACTGAAAAAGAACCCAAGAAGCTACCTGAAGGC
CCCGGGCCTGAGCAGAGAGTTCAAGACAAGCAGCTGATAGCACAAGGGGAGCCTGTG
CAGGAAGAGGATGAAGAGGAATTGAGGACCTTGCCTCGAGCTCCCCCCTCCCTGGAT
GGAGAAAACAGAGGCATCAGGACAAGGGACCCTGTCCTTCTGGCCCTCACAGGCCCT
CTGCTCATGGAGGGACTTGCTGATGCTGCCCTGGAAGATGTGGAGAACTTGCGTCAC
CTGATCCTGTGGAGCCTGCTGCCTGGTCACACTGTGAAGACTCAGGCTGCTGGCGAG
CCTGAGGATGACCTCACACCCACCCCTTCTGTCGTGAGCATCACCTCTCACCCCTGG
GACCCAGGGTCCCCAGGGCAAGCTCCCACCATAAGTGACAGCACCCGACTTGCGAGG
CCAGAGGGCAGCCAGCCAGAGGGCGAGGATGTTGCTGTCAGTTCTCTGGCACACCTG
CCGCCAAGGACCAGAAGTTCTGGCGTCTGGGACTCTCCTGAGCTGGATAGGAATCCG
GCTGCAGAGGCTGCAAGCACAGAACCAGCAGCAAGTTACAAGGTTGTGAGAAAAGTC
TCTCTACTCCCTGGTGGTGGTGTCGGTGCAGCCAAGGTGGCGGGCAGCAATGCTATC
CCTGACAGTGGCCAGTCAGAATCTGAGCTATCTGAAGTGGAAGGCGGAGCACAGGCT
ACGGGGAACTGTTTCTATGTCAGCATGCCAGCAGGACCTCTGGACTCCAGCACTGAG
CCTACTGGGACACCCCCAAGCCCCTCACAGTGTCACAGCCTCCCTGCATGGCCAACA
GAGCCTCAGCCCTACAGGGGAGTCCGTGGGGGTCAGTGTTCCAGCCTGGTCCGCAGG
GATGTGGATGTGATCTTCCATACCATCGAGCAGCTCACCATCAAGCTTCACAGACTG
AAGGACATGGAGCTGGCCCACAGAGAGCTGCTCAAGTCCCTTGGAGGAGAGTCATCG
GGTGGAACCACACCTGTGGGGAGTTTTCACACAGAGGCAGCCAGATGGACAGACTAC
TCCCTCTCTCCTCCAGCCAAGGAAGCCCTGGCCTCTGATTCCCAAAATGGTCAGGAG
CAGGGGTCCTGCCCTGAAGAAGGCTCCGACATCGCCCTGGAAGACAGTGCCACTGAC
ACAGCTGTGTCACCAGGACCATAG

FIGURE 17

MSIRLPHSIDRSASKKQSHLSSPIASWLSSLSSLGDSTPERTSPSHHRQPSDTSETTAG
LVQRCVIIQKDQHGFGFTVSGDRIVLVQSVRPGGAAMKAGVKEGDRIIKVNGTMVTNSS
HLEVVKLIKSGAYAALTLLGSSPPSVGVSGLQQNPSVAGVLRVNPIIPPPPPPPPLPPP
QHITGPKPLQDPEVQKHATQILWNMLRQEEEELQDILPPCGETSQRTCEGRLSVDSQEA
DSGLDSGTERFPSISESLMNRNSVLSDPGLDSPQTSPVILARVAQHHRRQGSDAALLPL
NHQGIDQSPKPLIIGPEEDYDPGYFNNESDIIFQDLEKLKSHPAYLVVFLRYILSQADP
GPLLFYLCSEVYQQTNPKDSRSLGKDIWNIFLEKNAPLRVKIPEMLQAEIDLRLRNNED
PRNVLCEAQEAVMLEIQEQINDYRSKRTLGLGSLYGENDLLGLDGDPLRERQMAEKQLA
ALGDILSKYEEDRSAPMDFAVNTFMSHAGIRLRESRSSCTAEKTQSAPDKDKWLPFFPK
TKKQSSNSKKEKDALEDKKRNPILRYIGKPKSSSQSIKPGNVRNIIQHFENSHQYDVPE
PGTQRLSTGSFPEDLLESDSSRSEIRLGRSGSLKGREEMKRSRKAENVPRPRSDVDMDA
AAEAARLHQSASSSASSLSTRSLENPTPPFTPKMGRRSIESPNLGFCTDVILPHLLEDD
LGQLSDLEPEPEVQNWQHTVGKDVVANLTQREIDRQEVINELFVTEASHLRTLRVLDLI
FYQRMRKENLMPREELARLFPNLPELIEIHNSWCEAMKKLREEGPIIRDISDPMLARFD
GPAREELQQVAAQFCSYQSVALELIRTKQRKESRFQLFMQEAESHPQCRRLQLRDLIVS
EMQRLTKYPLLLENIIKHTEGGTSEHEKLCRARDQCREILKFVNEAVKQTENRHRLEGY
QKRLDATALERASNPLAAEFKSLDLTTRKMIHEGPLTWRISKDKTLDLQVLLLEDLVVL
LQRQEERLLLKCHSKTAVGSSDSKQTFSPVLKLNAVLIRSVATDKRAFFIICTSELGPP
QIYELVALTSSDKNIWMELLEEAVQNATKHPGAAPIPIHPSPPGSQEPAYQGSTSSRVE
INDSEVYHTEKEPKKLPEGPGPEQRVQDKQLIAQGEPVQEEDEEELRTLPRAPPSLDGE
NRGIRTRDPVLLALTGPLLMEGLADAALEDVENLRHLILWSLLPGHTVKTQAAGEPEDD
LTPTPSVVSITSHPWDPGSPGQAPTISDSTRLARPEGSQPEGEDVAVSSLAHLPPRTRS
SGVWDSPELDRNPAAEAASTEPAASYKVVRKVSLLPGGGVGAAKVAGSNAIPDSGQSES
ELSEVEGGAQATGNCFYVSMPAGPLDSSTEPTGTPPSPSQCHSLPAWPTEPQPYRGVRG
GQCSSLVRRDVDVIFHTIEQLTIKLHRLKDMELAHRELLKSLGGESSGGTTPVGSFHTE
AARWTDYSLSPPAKEALASDSQNGQEQGSCPEEGSDIALEDSATDTAVSPGP.

FIGURE 18

ATGGACGTGAACCTTGCCCCGCTCCGTGCCTGGGATGATTTCTTCCCGGGCTCTGATCG
TTTCGCACGGCCGGACTTCAGGGATATATCCAAATGGAACAACCGTGTAGTGAGCAATC
TGCTCTATTACCAGACCAACTACCTGGTGGTGGCTGCCATGATGATTTCAGTCGTTGGG
TTTCTGAGCCCCTTCAACATGATCCTTGGAGGAATCATTGTGGTGCTGGTGTTCACGGG
GTTTGTGTGGGCAGCACACAATAAAGACATCCTCCGCCGGATGAAGAAGCAGTACCCAA
CGGCCTTTGTCATGGTGGTCATGCTAGCCAGCTACTTCCTCATATCCATGTTTGGGGGT
GTCATGGTCTTTGTGTTTGGCATCACGTTTCCCTTATTGTTGATGTTCATCCATGCATC
CCTGAGACTTCGAAACCTCAAGAACAAACTGGAAAATAAAATGGAGGGAATAGGCTTGA
AGAAAACGCCGATGGGCATCATCCTGGATGCCTTGGAACAGCAGGAAGACAGCATCAAT
AAATTTGCTGACTACATCAGCAAAGCCAGGGAGTAA

FIGURE 19

MDVNLAPLRAWDDFFPGSDRFARPDFRDISKWNNRVVSNLLYYQTNYLVVAAMMISVVG
FLSPFNMILGGIIVVLVFTGFVWAAHNKDILRRMKKQYPTAFVMVVMLASYFLISMFGG
VMVFVFGITFPLLLMFIHASLRLRNLKNKLENKMEGIGLKKTPMGIILDALEQQEDSIN
KFADYISKARE

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FIGURE 20A

1 gttggccacc atggggatgt accaagtgag actgtaggga aagaaggtgg tgactcgcgt
61 gcctggctac tggctgctgc tcacctcgat gctacaagat tcctagcaag atcaaaactg
121 accattaacc tacctctaca tccccctggc gccgttccag ggccaacgcc acattccctg
181 ctgggcacgc aatggccgca ccccctcccg ctacagaagg ctcttttggt acacgcagtc
241 cgaggtcgcc atggatcgga tgaagaagat caaacggcag ctgtcaatga cactccgagg
301 gggccgaggc atagacaaga ccaatggtgt ccctgagcag ataggcctag atgagagtgg
361 tggtggtggt ggcatgaccc ttggagaagc tcccacccgt gttgcccctg gggaacttcg
421 ctctattcgg ggcccactca gctctgcacc aggtctacct gggtttccca gtctgctcta
481 ggggccatgt acacaaatgg atacgatgaa gaaatatatt atattggggg aaagagagtg
541 ttcttgactc caaaggcctg gcctttccct cactctgcac cagagattgt gcatgaagac
601 atgaagatgg gatctgatgg ggagagtgac caggcttcag ccacatcctc agatgaggtg
661 cagtctccag tgagagtgcg catgcgcaac caccccccac gcaagatctc cactgaggat
721 atcaacaaat gcctgtcact accagctgac atacggctgc ctgagggcta ccttgagaag
781 ctgaccctca atagccccat cggtgataag cctcttagcc ggcgcctccg gccagtcagc
841 ttgtctgaga ttggctttgg aaaactggag acctacatca aactagacaa gctgggtgag
901 ggtacctatg ccactgtcta caaaggcaaa agcaagctca cagacaacct tgtagcactt
961 aaggagatca gactggaaca cgaagaaggg gcaccctgca ctgctatccg ggaagtatcc
1021 ctgcttaagg acctcaagca tgccaacatc gtcacactac atgacattat ccacacagag
1081 aagtccctca cccttgtctt tgaatacttg gacaaggacc tgaagcagta cctggatgac
1141 tgtggaaatg tcatcaacat gcacaatgtg aaactgttcc tgttccagtt gctccgtggc
1201 ctggcctact gccacaggca gaaggtgcta caccgagacc tcaagcccca gaacctactc
1261 atcaacgaga ggggagagct caaactggca gactttggcc tggcttacgc caagtcaatt
1321 cctactaaaa catactccaa cgaagtggtg acactgtggt accggccccc tgacatctta
1381 cttgggtcca cagactactc cggccaaatt gacatgtggg gtgttggctg catcttttat
1441 gagatggcca caggccggcc cctcttccca ggctccacag tggaagaaca gctgcacttc
1501 atcttccgca ttttgggaac cccaactgag gacacatggc caggtatcct gtccaatgaa
1561 gagtttagaa catacaacta ccccaagtac cgagccgagg cccttctgag gcatgcaccc
1621 cgacttgaat gcgatggagc tgacctcctc accaagctgc tgcagtttga gggtcgcaat
1681 cggatctctg ctgaggatgc catgaaacat ccattctttc tcagcttggg ggagcggatc
1741 cacaaacttc ctgacactac ttccatattt gcactaaagg aggtacagct acaaaaggag
1801 gccaacattc ggtccacttc tatgcctgac tcaggcaggc cagctttccg tgtggtggat
1861 accgagttct aagccaagtt ttaagccaca gacagaccaa ggccccagca ggcagcggct
1921 ggagggatgc cacacccctc acaggacagc ccccatctgc aatcctccct gcttgttgcc
1981 tgcttacctg cctgagccac actcccctgc caacttgtcc cctgccacct gtccaaacac
2041 cgaactactg gcctggcctg tcaacccaac cactggcctg tctgctgggt gctaacaaag
2101 ctctcaccac tactttgctt gatgtgtctg tctctgtctt ggtagatgct ggtggaccga
2161 atggccgtgc cagctttcca cactaaggct aggccttccc ctcttcatca cactctctcc
2221 caggaccact accccatggc cagccagggg tttggagcta gcccaggcca ggctcttaat
2281 cgactttgac tagaaggtag tgagtgatgc cttgggtctg agcatcattt gcctgcttcc
2341 cacctgtccc acttgcctct gttgtatggg ctttttttta gtttctttta ttgttttttt
2401 attattttaa atgaggttct cacttttaa tgcaatatct ctgtatacag actggttggg

FIGURE 20B

2461 cactactccc tgagtgtggc actcccacag tattttgtgc aatgaagtcc cactcccacc
2521 ctttgagagg tagggaccca gaccctattc agatcctcac catcactaga ccctggaatt
2581 ggctatggga aagcatgcct cagccactca ccttcctccc ctacctagcg ttcccagcta
2641 taggggggacc tgagaactac cagagagtgg gagatggaca tggtggggcc tacttttcc
2701 ctccttcagt cccgtagcca gggcctcctt ccttctcagg gtcttcccca gcccagctct
2761 gcctagccct cctgccctgt cctactcggt gctgttgagt aggggctctg cctggaatcg
2821 agcagcttag tgaggagcca tatataatat gtgcacaagc aggaggacat gtgggagctt
2881 gtgcccaatt gttacacccc aatccctagg agggtcaggc aggccaagga cagtctcctg
2941 gatggatggt ttgctccct tactccacct taagccttgg gacccttaag cagggtggga
3001 gggcaaggga gggtgccctc ctagtggggt ttgggggat tgggttcctg aatgcaccat
3061 aatcgctgta tgaaatatta aaaaaaagtc taaagtgaaa aaaaaaaaaa a

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FIGURE 21

MDRMKKIKRQLSMTLRGGRGIDKTNGVPEQIGLDESGGGGGMTLGEAPTRVAPGELRSI
RGPLSSAPEIVHEDMKMGSDGESDQASATSSDEVQSPVRVRMRNHPPRKISTEDINKCL
SLPADIRLPEGYLEKLTLNSPIGDKPLSRRLRPVSLSEIGFGKLETYIKLDKLGEGTYA
TVYKGKSKLTDNLVALKEIRLEHEEGAPCTAIREVSLLKDLKHANIVTLHDIIHTEKSL
TLVFEYLDKDLKQYLDDCGNVINMHNVKLFLFQLLRGLAYCHRQKVLHRDLKPQNLLIN
ERGELKLADFGLAYAKSIPTKTYSNEVVTLWYRPPDILLGSTDYSGQIDMWGVGCIFYE
MATGRPLFPGSTVEEQLHFIFRILGTPTEDTWPGILSNEEFRTYNYPKYRAEALLRHAP
RLECDGADLLTKLLQFEGRNRISAEDAMKHPFFLSLGERIHKLPDTTSIFALKEVQLQK
EANIRSTSMPDSGRPAFRVVDTEF

FIGURE 22

MYTNGYDEEIYYIGGKRVFLTPKAWPFFPHSAPEIVHEDMKMGSDGESDQASATSSDEVQ
SPVRVRMRNHPPRKISTEDINKCLSLPADIRLPEGYLEKLTLNSPIGDKPLSRRLRPVS
LSEIGFGKLETYIKLDKLGEGTYATVYKGKSKLTDNLVALKEIRLEHEEGAPCTAIREV
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LGERIHKLPDTTSIFALKEVQLQKEANIRSTSMPDSGRPAFRVVDTEF

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship is as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **GLUTAMATE TRANSPORTER ASSOCIATED PROTEINS AND METHODS OF USE THEREOF** the specification of which

__X__ is attached hereto.

_____ was filed on __**October 23, 2000**__, (Attorney Docket No.: JHU1650-2) as U.S. Application Serial No. _____ and was amended on _____ if applicable (the "Application").

I hereby authorize and request insertion of the application serial number of the Application when officially known.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability of the subject matter of the Application as defined in Title 37, Code of Federal Regulations ("C.F.R."), § 1.56.

With respect to the Application, I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/161,007	October 23, 1999
(Application Serial No.)	(Filing Date)
60/206,157	May 22, 2000
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

With respect to the Application, I hereby claim the benefit under 35 U.S.C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of the application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability of the subject matter of the Application as defined in Title 37, C.F.R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of the Application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature: _____

Date: _____

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PATENT
ATTORNEY DOCKET NO.: JHU1650-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Rothstein, et al.	Art Unit:	
Application No.:		Examiner:	
Filed:	Herewith		
Title:	**GLUTAMATE TRANSPORTER ASSOCIATED PROTEINS AND METHODS OF USE THEREOF**		

ATTN: BOX PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

POWER OF ATTORNEY BY ASSIGNEE

As a below-named assignee of the above-identified application ("Application"):

I hereby appoint the following attorneys of the assignee to prosecute the Application and to transact all business in the United States Patent and Trademark Office connected therewith:

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Docket #JHU1650-2

I hereby authorize and request insertion of the application number of the Application when officially known.

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By: _____

Name: _____

Title: _____

Date: _____

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Law, Robert
Orlov, Irina

<120> GLUTAMATE TRANSPORTER ASSOCIATED PROTEINS AND METHODS OF USE THEREOF

<130> JHU1650-2

<150> 60/161,007
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Met Ser Ser Thr Leu Ser Pro Thr Asp Phe Asp Ser Leu Glu Ile Gln
1               5                   10                  15

ggc cag tac agt gac atc aac aac cgc tgg gac ctg ccc gac tca gat       96
Gly Gln Tyr Ser Asp Ile Asn Asn Arg Trp Asp Leu Pro Asp Ser Asp
            20                  25                  30

tgg gac aat gac agc agt tca gcc cgc ctc ttt gag agg tcc aga att      144
Trp Asp Asn Asp Ser Ser Ser Ala Arg Leu Phe Glu Arg Ser Arg Ile
        35                  40                  45

aag gcc ctg gca gat gag cga gaa gcc gtg cag aag aaa acc ttc acc      192
Lys Ala Leu Ala Asp Glu Arg Glu Ala Val Gln Lys Lys Thr Phe Thr
    50                  55                  60

aag tgg gtg aac tcc cac ctg gcc cgg gtg aca tgc cgg gtg gga gac      240
Lys Trp Val Asn Ser His Leu Ala Arg Val Thr Cys Arg Val Gly Asp
65                  70                  75                  80

ctg tac agc gac ctg cgg gac ggg cgc aac ctc ctg agg ctc ctg gag      288
Leu Tyr Ser Asp Leu Arg Asp Gly Arg Asn Leu Leu Arg Leu Leu Glu
                85                  90                  95

gtg ctc tcg gga gag acc ctg cca aaa ccc acc aag ggc cgg atg cgg      336
Val Leu Ser Gly Glu Thr Leu Pro Lys Pro Thr Lys Gly Arg Met Arg
            100                 105                 110

att cac tgc ctg gag aat gtc gac aaa gca ctg cag ttc ctg aag gag      384
Ile His Cys Leu Glu Asn Val Asp Lys Ala Leu Gln Phe Leu Lys Glu
        115                 120                 125

cag aag gtg cac ctg gaa aac atg ggc tcc cac gac att gtg gat ggg      432
Gln Lys Val His Leu Glu Asn Met Gly Ser His Asp Ile Val Asp Gly
    130                 135                 140
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aac cac cgt ctg acc ctt ggg cta gtg tgg acc atc atc ctc cga ttt      480
Asn His Arg Leu Thr Leu Gly Leu Val Trp Thr Ile Ile Leu Arg Phe
145                 150                 155                 160

cag atc caa gac atc agt gtg gag aca gaa gac aac aag gag aag aag      528
Gln Ile Gln Asp Ile Ser Val Glu Thr Glu Asp Asn Lys Glu Lys Lys
                165                 170                 175

tca gcc aag gat gcc ctg ctg ctg tgg tgc cag atg aag act gca ggg      576
Ser Ala Lys Asp Ala Leu Leu Leu Trp Cys Gln Met Lys Thr Ala Gly
            180                 185                 190

tat ccc aat gtc aat gtg cac aac ttt acc acc agt tgg aga gat ggg      624
Tyr Pro Asn Val Asn Val His Asn Phe Thr Thr Ser Trp Arg Asp Gly
        195                 200                 205

ctg gcc ttt aat gcc att gtg cac aaa cac cgg cca gac ctg ttg gat      672
Leu Ala Phe Asn Ala Ile Val His Lys His Arg Pro Asp Leu Leu Asp
    210                 215                 220

ttt gag tcc ctg aag aag tgt aac gca cac tac aat ctg cag aat gct      720
Phe Glu Ser Leu Lys Lys Cys Asn Ala His Tyr Asn Leu Gln Asn Ala
225                 230                 235                 240

ttc aat ctg gct gaa aag gaa ctt ggc ctg acg aag ctc ctg gat cct      768
Phe Asn Leu Ala Glu Lys Glu Leu Gly Leu Thr Lys Leu Leu Asp Pro
                245                 250                 255

gaa gat gtg aac gta gac caa ccc gat gag aag tcc atc atc acc tac      816
Glu Asp Val Asn Val Asp Gln Pro Asp Glu Lys Ser Ile Ile Thr Tyr
            260                 265                 270

gtg gcc act tac tac cac tac ttc tcg aag atg aag gcc ctg gct gtg      864
Val Ala Thr Tyr Tyr His Tyr Phe Ser Lys Met Lys Ala Leu Ala Val
        275                 280                 285

gaa ggc aaa agg att ggc aag gtc ctg gac cat gcc atg gag gca gaa      912
Glu Gly Lys Arg Ile Gly Lys Val Leu Asp His Ala Met Glu Ala Glu
    290                 295                 300

cac ctg gta gag aaa tat gag tcc ctg gcc tct gaa ctg ctg cag tgg      960
His Leu Val Glu Lys Tyr Glu Ser Leu Ala Ser Glu Leu Leu Gln Trp
305                 310                 315                 320

atc gag caa acg att ggg acc ttc aat gac cga cag ctg gcc aac tcc     1008
Ile Glu Gln Thr Ile Gly Thr Phe Asn Asp Arg Gln Leu Ala Asn Ser
                325                 330                 335

ctg agt ggc gtc cag aac cag ctg cag tct ttc aat tcc tac cgc acg     1056
Leu Ser Gly Val Gln Asn Gln Leu Gln Ser Phe Asn Ser Tyr Arg Thr
            340                 345                 350

gtg gag aag cca ccc aag ttc aca gag aaa ggg aac ttg gag gtg ttg     1104
Val Glu Lys Pro Pro Lys Phe Thr Glu Lys Gly Asn Leu Glu Val Leu
        355                 360                 365

ctc ttc acc atc cag agt aag ctg cgg gcc aac aac cag aaa gtc tac     1152
Leu Phe Thr Ile Gln Ser Lys Leu Arg Ala Asn Asn Gln Lys Val Tyr
    370                 375                 380

aca cca cgc gaa ggc cgg ctc atc tcg gac atc aac aag gcc tgg gag     1200
Thr Pro Arg Glu Gly Arg Leu Ile Ser Asp Ile Asn Lys Ala Trp Glu
385                 390                 395                 400
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cgg cta gag aaa gcc gaa cat gag cga gag ctg gcc ctg cgc acg gag     1248
Arg Leu Glu Lys Ala Glu His Glu Arg Glu Leu Ala Leu Arg Thr Glu
                405                 410                 415

ctg atc cgc cag gag aag ctg gag caa ctg gct gct cgc ttc gac cgc     1296
Leu Ile Arg Gln Glu Lys Leu Glu Gln Leu Ala Ala Arg Phe Asp Arg
            420                 425                 430

aag gct gcc atg cgg gag acc tgg ctc agt gag aac cag cgc ctc gtc     1344
Lys Ala Ala Met Arg Glu Thr Trp Leu Ser Glu Asn Gln Arg Leu Val
        435                 440                 445

tcc cag gac aac ttt ggc ctg gag ctg gca gca gtg gag gca gca gtg     1392
Ser Gln Asp Asn Phe Gly Leu Glu Leu Ala Ala Val Glu Ala Ala Val
    450                 455                 460

cgg aag cat gaa gcc att gag aca gac att gtg gcc tac agc ggc cgg     1440
Arg Lys His Glu Ala Ile Glu Thr Asp Ile Val Ala Tyr Ser Gly Arg
465                 470                 475                 480

gtg caa gcg gtg gac gcc gta gcc gca gaa ctg gcc gct gag cat tac     1488
Val Gln Ala Val Asp Ala Val Ala Ala Glu Leu Ala Ala Glu His Tyr
                485                 490                 495

cat gac att aag cgc att gcg gcg cgg cag aac aac gtg gcc cgg ctc     1536
His Asp Ile Lys Arg Ile Ala Ala Arg Gln Asn Asn Val Ala Arg Leu
            500                 505                 510

tgg gac ttc tta cga gag atg gtg gcc gcc cgc cgt gag cgg ctc ctt     1584
Trp Asp Phe Leu Arg Glu Met Val Ala Ala Arg Arg Glu Arg Leu Leu
        515                 520                 525

ctc aac ctg gag ctg cag aag gtg ttt cag gac ctg ctc tac ctc atg     1632
Leu Asn Leu Glu Leu Gln Lys Val Phe Gln Asp Leu Leu Tyr Leu Met
    530                 535                 540

gac tgg atg gca gag atg aag ggc cgg ctg cag tct cag gac cta ggc     1680
Asp Trp Met Ala Glu Met Lys Gly Arg Leu Gln Ser Gln Asp Leu Gly
545                 550                 555                 560

aag cat ctg gct gga gtg gaa gat cta ctg caa cta cac gaa ctg gtg     1728
Lys His Leu Ala Gly Val Glu Asp Leu Leu Gln Leu His Glu Leu Val
                565                 570                 575

gag gcg gac att gca gtt cag gct gag agg gtg cga gcg gtc agc gcc     1776
Glu Ala Asp Ile Ala Val Gln Ala Glu Arg Val Arg Ala Val Ser Ala
            580                 585                 590

tct gcc ctg cgc ttc tgc gac cca ggg aaa gag tat aga cct tgc ggc     1824
Ser Ala Leu Arg Phe Cys Asp Pro Gly Lys Glu Tyr Arg Pro Cys Gly
        595                 600                 605

ccg cag cta gtg tca gag agg gta gcc act ctg gag cag agc tat gag     1872
Pro Gln Leu Val Ser Glu Arg Val Ala Thr Leu Glu Gln Ser Tyr Glu
    610                 615                 620

gcc ctg tgc gaa ttg gca gca act cga agg gcc cga ctg gaa gag tcc     1920
Ala Leu Cys Glu Leu Ala Ala Thr Arg Arg Ala Arg Leu Glu Glu Ser
625                 630                 635                 640

cgt cgt ctc tgg agg ttc ctc tgg gaa gtg ggt gag gcc gag gcc tgg     1968
Arg Arg Leu Trp Arg Phe Leu Trp Glu Val Gly Glu Ala Glu Ala Trp
                645                 650                 655

gtt cgg gag cag cag cac ctc ctg gcc tca gct gag aca ggc cgg gac     2016
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Val Arg Glu Gln Gln His Leu Leu Ala Ser Ala Glu Thr Gly Arg Asp
            660                 665                 670

ctg act ggt gtc ctc cgc ctg ctc aat aag cac aca gcc cta cgg ggt     2064
Leu Thr Gly Val Leu Arg Leu Leu Asn Lys His Thr Ala Leu Arg Gly
        675                 680                 685

gag atg agt ggc cgc ctg ggg ccc ctg aag ctc acc ctg gaa caa ggt     2112
Glu Met Ser Gly Arg Leu Gly Pro Leu Lys Leu Thr Leu Glu Gln Gly
    690                 695                 700

cag cag tta gtt gcc gag ggc cac cct gga gct aac caa gcc tca acc     2160
Gln Gln Leu Val Ala Glu Gly His Pro Gly Ala Asn Gln Ala Ser Thr
705                 710                 715                 720

cgt gca gca gag ctc cag gcc cag tgg gag cga cta gaa gcc ctg gcc     2208
Arg Ala Ala Glu Leu Gln Ala Gln Trp Glu Arg Leu Glu Ala Leu Ala
                725                 730                 735

gag gag cga gcc cag cgg cta gca cag gct gcc agc ctc tac cag ttc     2256
Glu Glu Arg Ala Gln Arg Leu Ala Gln Ala Ala Ser Leu Tyr Gln Phe
            740                 745                 750

cag gca gat gcc aat gac atg gag gct tgg ttg gtg gac gca cta cgc     2304
Gln Ala Asp Ala Asn Asp Met Glu Ala Trp Leu Val Asp Ala Leu Arg
        755                 760                 765

ctg gta tct agc cct gag gta ggg cac gat gag ttc tcc acg cag gcc     2352
Leu Val Ser Ser Pro Glu Val Gly His Asp Glu Phe Ser Thr Gln Ala
    770                 775                 780

ctg gcc agg cag cac agg gcc ctt gag gag gag atc cga gcc cac cgg     2400
Leu Ala Arg Gln His Arg Ala Leu Glu Glu Glu Ile Arg Ala His Arg
785                 790                 795                 800

cct aca ctg gat gcc ttg agg gag cag gct gca gcc ctg cca cct gca     2448
Pro Thr Leu Asp Ala Leu Arg Glu Gln Ala Ala Ala Leu Pro Pro Ala
                805                 810                 815

ctg agc cac aca cct gag gta cag ggc agg gtg ccc act ctg gag cag     2496
Leu Ser His Thr Pro Glu Val Gln Gly Arg Val Pro Thr Leu Glu Gln
            820                 825                 830

cac tat gag gag ctg cag gcc cgg gca ggt gag cgt gca cga gcc ctg     2544
His Tyr Glu Glu Leu Gln Ala Arg Ala Gly Glu Arg Ala Arg Ala Leu
        835                 840                 845

gaa gca gcc ctg gcg ttc tat acc atg ctc agc gag gcc ggg gct tgt     2592
Glu Ala Ala Leu Ala Phe Tyr Thr Met Leu Ser Glu Ala Gly Ala Cys
    850                 855                 860

ggg ctc tgg gta gag gag aag gag cag tgg ctc aac ggg ctg gcc cta     2640
Gly Leu Trp Val Glu Glu Lys Glu Gln Trp Leu Asn Gly Leu Ala Leu
865                 870                 875                 880

cct gag cgc ctg gag gac ccg gag gtg gtc caa cag agg ttt gag acc     2688
Pro Glu Arg Leu Glu Asp Pro Glu Val Val Gln Gln Arg Phe Glu Thr
                885                 890                 895

tta gag ccc gaa atg aac gcc ctg gct gca cgg att act gct gtc agt     2736
Leu Glu Pro Glu Met Asn Ala Leu Ala Ala Arg Ile Thr Ala Val Ser
            900                 905                 910

gac ata gct gag cag ttg ctg aag gcc agt cca cca ggc aag gac cgc     2784
Asp Ile Ala Glu Gln Leu Leu Lys Ala Ser Pro Pro Gly Lys Asp Arg
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        915                 920                 925

atc att ggc acc cag gag cag ctc aac caa agg tgg cag cag ttc agg     2832
Ile Ile Gly Thr Gln Glu Gln Leu Asn Gln Arg Trp Gln Gln Phe Arg
    930                 935                 940

tcc ctg gca ggt ggc aaa aag gca gct ctg aca tca gcc ctg agc atc     2880
Ser Leu Ala Gly Gly Lys Lys Ala Ala Leu Thr Ser Ala Leu Ser Ile
945                 950                 955                 960

cag aat tac cac cta gag tgc aca gag acc cag gcc tgg atg aga gaa     2928
Gln Asn Tyr His Leu Glu Cys Thr Glu Thr Gln Ala Trp Met Arg Glu
                965                 970                 975

aag acc aag gtc att gag tct acc cag gac cta ggc aat gat cta gct     2976
Lys Thr Lys Val Ile Glu Ser Thr Gln Asp Leu Gly Asn Asp Leu Ala
            980                 985                 990

ggt gtg ctg gcc ctg cag cgg aag  ctg gca ggc act gag  aga gat ctg   3024
Gly Val Leu Ala Leu Gln Arg Lys  Leu Ala Gly Thr Glu  Arg Asp Leu
        995                 1000                1005

gaa gcc  atc tct gcc cgg gtg  ggt gag ctg acc caa  gag gca aat      3069
Glu Ala  Ile Ser Ala Arg Val  Gly Glu Leu Thr Gln  Glu Ala Asn
    1010                1015                1020

gct ttg  gct gct ggg cac cca  gcc caa gcc cct gcc  atc aac aca      3114
Ala Leu  Ala Ala Gly His Pro  Ala Gln Ala Pro Ala  Ile Asn Thr
    1025                1030                1035

cgg ctt  gga gag gtt caa act  gga tgg gag gat ctt  cgg gca acc      3159
Arg Leu  Gly Glu Val Gln Thr  Gly Trp Glu Asp Leu  Arg Ala Thr
    1040                1045                1050

atg agg  cgg aga gaa gag tcc  ctg ggt gag gct cga  cgg ctg caa      3204
Met Arg  Arg Arg Glu Glu Ser  Leu Gly Glu Ala Arg  Arg Leu Gln
    1055                1060                1065

gat ttc  ctg cgc agc tta gat  gac ttc cag gcc tgg  cta ggc cgc      3249
Asp Phe  Leu Arg Ser Leu Asp  Asp Phe Gln Ala Trp  Leu Gly Arg
    1070                1075                1080

aca cag  act gct gta gcc tct  gag gaa gga cca gcc  acc ctt cca      3294
Thr Gln  Thr Ala Val Ala Ser  Glu Glu Gly Pro Ala  Thr Leu Pro
    1085                1090                1095

gag gca  gaa gcc ctc tta gcc  cag cat gca gct ctg  cgg gga gag      3339
Glu Ala  Glu Ala Leu Leu Ala  Gln His Ala Ala Leu  Arg Gly Glu
    1100                1105                1110

gtg gag  aga gcc cag agc gag  tac agc cgc ctc agg  acc ttg ggc      3384
Val Glu  Arg Ala Gln Ser Glu  Tyr Ser Arg Leu Arg  Thr Leu Gly
    1115                1120                1125

gag gag  gtg acc aga gac cag  gct gat ccc caa tgc  ctc ttc ctc      3429
Glu Glu  Val Thr Arg Asp Gln  Ala Asp Pro Gln Cys  Leu Phe Leu
    1130                1135                1140

aga cag  agg ctg gaa gcc ctt  gga acc ggc tgg gag  gag ctg ggt      3474
Arg Gln  Arg Leu Glu Ala Leu  Gly Thr Gly Trp Glu  Glu Leu Gly
    1145                1150                1155

cgc atg  tgg gag agc cgg caa  ggc cgc ttg gcc caa  gcc cat ggc      3519
Arg Met  Trp Glu Ser Arg Gln  Gly Arg Leu Ala Gln  Ala His Gly
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agc agc  cag gaa tat gtt ctg  tct cac acg gag atg  cca ggg aca      3609
Ser Ser  Gln Glu Tyr Val Leu  Ser His Thr Glu Met  Pro Gly Thr
    1190                 1195                 1200

ctg cag  gcg gcg gat gca gcc  att aaa aag ctg gaa  gac ttc atg      3654
Leu Gln  Ala Ala Asp Ala Ala  Ile Lys Lys Leu Glu  Asp Phe Met
    1205                 1210                 1215

agc acc  atg gac gcc aat gga  gag cgc atc cgt gga  ctc ctg gag      3699
Ser Thr  Met Asp Ala Asn Gly  Glu Arg Ile Arg Gly  Leu Leu Glu
    1220                 1225                 1230

gct ggc  cgt cag ctg gtg tcc  aag ggc aat atc cat  gct gag aag      3744
Ala Gly  Arg Gln Leu Val Ser  Lys Gly Asn Ile His  Ala Glu Lys
    1235                 1240                 1245

atc caa  gag aag gca gac tcc  atc gag aag agg cac  aga aag aac      3789
Ile Gln  Glu Lys Ala Asp Ser  Ile Glu Lys Arg His  Arg Lys Asn
    1250                 1255                 1260

cag gag  gcc gtg cag cag ctt  cta ggc cgc ctt cgg  gac aac cga      3834
Gln Glu  Ala Val Gln Gln Leu  Leu Gly Arg Leu Arg  Asp Asn Arg
    1265                 1270                 1275

gag cag  cag cac ttc ttg caa  gac tgt cag gag ctg  aaa ctc tgg      3879
Glu Gln  Gln His Phe Leu Gln  Asp Cys Gln Glu Leu  Lys Leu Trp
    1280                 1285                 1290

att gac  gag aag atg ctg aca  gct cag gat gtg tcc  tat gat gag      3924
Ile Asp  Glu Lys Met Leu Thr  Ala Gln Asp Val Ser  Tyr Asp Glu
    1295                 1300                 1305

gca cgc  aac ctg cac acc aag  tgg caa aaa cac cag  gca ttc atg      3969
Ala Arg  Asn Leu His Thr Lys  Trp Gln Lys His Gln  Ala Phe Met
    1310                 1315                 1320

gcc gag  ctg gca gcc aac aag  gac tgg ctg gac aaa  gtg gac aag      4014
Ala Glu  Leu Ala Ala Asn Lys  Asp Trp Leu Asp Lys  Val Asp Lys
    1325                 1330                 1335

gaa ggg  cgg gag ctg act ctt  gaa aag cca gaa ctc  aaa gtc cta      4059
Glu Gly  Arg Glu Leu Thr Leu  Glu Lys Pro Glu Leu  Lys Val Leu
    1340                 1345                 1350

gtg tca  gag aag ctg gag gac  ctg cac agg cgc tgg  gat gaa ctg      4104
Val Ser  Glu Lys Leu Glu Asp  Leu His Arg Arg Trp  Asp Glu Leu
    1355                 1360                 1365

gag act  acc acc caa gcc aag  gcc cgc agt ctt ttt  gat gct aac      4149
Glu Thr  Thr Thr Gln Ala Lys  Ala Arg Ser Leu Phe  Asp Ala Asn
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cgg gca  gag cta ttt gcc caa  agc tgt tct gcc ctg  gag agc tgg      4194
Arg Ala  Glu Leu Phe Ala Gln  Ser Cys Ser Ala Leu  Glu Ser Trp
    1385                 1390                 1395

ctg gag  agc ctg cag gcc cag  ctg cac tca gat gac  tat ggc aag      4239
Leu Glu  Ser Leu Gln Ala Gln  Leu His Ser Asp Asp  Tyr Gly Lys
    1400                 1405                 1410
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gac ctc  acc agt gtc aac att  ctg cta aag aag caa  cag atg ctg      4284
Asp Leu  Thr Ser Val Asn Ile  Leu Leu Lys Lys Gln  Gln Met Leu
    1415                 1420                 1425

gaa cga  gag atg gct gtg aga  gag aag gag gta gag  gct atc cag      4329
Glu Arg  Glu Met Ala Val Arg  Glu Lys Glu Val Glu  Ala Ile Gln
    1430                 1435                 1440

gcc cag  gca aaa gcc ctg gcc  cag gaa gac caa agt  gca gga gag      4374
Ala Gln  Ala Lys Ala Leu Ala  Gln Glu Asp Gln Ser  Ala Gly Glu
    1445                 1450                 1455

gtg gaa  agg acc tcc aga gct  gtg gag gag aag ttc  agg gcc ttg      4419
Val Glu  Arg Thr Ser Arg Ala  Val Glu Glu Lys Phe  Arg Ala Leu
    1460                 1465                 1470

tgt cag  ccc atg aag gac cgc  tgc cgg cgc ctg caa  gcc tcc cga      4464
Cys Gln  Pro Met Lys Asp Arg  Cys Arg Arg Leu Gln  Ala Ser Arg
    1475                 1480                 1485

gag cag  cac cag ttc cac cgg  gat gtg gag gat gag  ata ctg tgg      4509
Glu Gln  His Gln Phe His Arg  Asp Val Glu Asp Glu  Ile Leu Trp
    1490                 1495                 1500

gtg acc  gag cgg ctt ccc atg  gcc agc tct ctg gag  cat ggc aag      4554
Val Thr  Glu Arg Leu Pro Met  Ala Ser Ser Leu Glu  His Gly Lys
    1505                 1510                 1515

gac ttg  ccc agc gtc cag ctt  ctc atg aag aaa aac  cag act ctg      4599
Asp Leu  Pro Ser Val Gln Leu  Leu Met Lys Lys Asn  Gln Thr Leu
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cag aag  gag atc cag ggc cat  gag ccc cgg att gca  gac ctc aaa      4644
Gln Lys  Glu Ile Gln Gly His  Glu Pro Arg Ile Ala  Asp Leu Lys
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gag agg  cag cgc act ctg aga  aca gca gca gcg ggt  cca gag ctg      4689
Glu Arg  Gln Arg Thr Leu Arg  Thr Ala Ala Ala Gly  Pro Glu Leu
    1550                 1555                 1560

gct gag  ctc cag gaa atg tgg  aaa cgc ctg agc cat  gag ctg gag      4734
Ala Glu  Leu Gln Glu Met Trp  Lys Arg Leu Ser His  Glu Leu Glu
    1565                 1570                 1575

ctt cgg  ggt aaa cga ctg gag  gag gcc ctt cga gcc  cag caa ttc      4779
Leu Arg  Gly Lys Arg Leu Glu  Glu Ala Leu Arg Ala  Gln Gln Phe
    1580                 1585                 1590

tat cgt  gac gct gca gag gcc  gag gct tgg atg ggg  gag cag gag      4824
Tyr Arg  Asp Ala Ala Glu Ala  Glu Ala Trp Met Gly  Glu Gln Glu
    1595                 1600                 1605

tta cat  atg atg ggc cag gaa  aag gcc aag gat gag  ctg agc gcc      4869
Leu His  Met Met Gly Gln Glu  Lys Ala Lys Asp Glu  Leu Ser Ala
    1610                 1615                 1620

cag gca  gaa gtg aag aag cat  cag gta cta gaa caa  gcc ctt gct      4914
Gln Ala  Glu Val Lys Lys His  Gln Val Leu Glu Gln  Ala Leu Ala
    1625                 1630                 1635

gac tat  gcc cag acc atc aaa  caa cta gca gcc agc  agt caa gat      4959
Asp Tyr  Ala Gln Thr Ile Lys  Gln Leu Ala Ala Ser  Ser Gln Asp
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atg att  gac cat gaa cat cca  gag agc aca agg tta  aca ata cgc      5004
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Replacement Sheet

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Met Ile  Asp His Glu His Pro  Glu Ser Thr Arg Leu  Thr Ile Arg
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caa gcc  cag gtg gac aag ctg  tac gcc ggc cta aag  gag ctg gca      5049
Gln Ala  Gln Val Asp Lys Leu  Tyr Ala Gly Leu Lys  Glu Leu Ala
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gga gag  cgg cgt gag cgt ctg  cag gag cac ctc agg  ctg tgc cag      5094
Gly Glu  Arg Arg Glu Arg Leu  Gln Glu His Leu Arg  Leu Cys Gln
    1685                 1690                 1695

ctc cgc  aga gag ctg gat gac  ctg gag cag tgg ata  cag gag cga      5139
Leu Arg  Arg Glu Leu Asp Asp  Leu Glu Gln Trp Ile  Gln Glu Arg
    1700                 1705                 1710

gaa gtc  gtg gca gcc tcc cat  gaa ctg ggc cag gac  tat gag cat      5184
Glu Val  Val Ala Ala Ser His  Glu Leu Gly Gln Asp  Tyr Glu His
    1715                 1720                 1725

gtg act  atg ctt cgg gac aaa  ttc cga gag ttc tcc  agg gac acc      5229
Val Thr  Met Leu Arg Asp Lys  Phe Arg Glu Phe Ser  Arg Asp Thr
    1730                 1735                 1740

agc acc  att ggc caa gag cgt  gta gac agt gcc aat  gcc ctg gcc      5274
Ser Thr  Ile Gly Gln Glu Arg  Val Asp Ser Ala Asn  Ala Leu Ala
    1745                 1750                 1755

aat ggg  ctc att gct ggg ggc  cat gct gca tgg gcc  acc gtg gcc      5319
Asn Gly  Leu Ile Ala Gly Gly  His Ala Ala Trp Ala  Thr Val Ala
    1760                 1765                 1770

gag tgg  aag gac agt ctc aat  gag gcc tgg gct gac  ctg ctg gag      5364
Glu Trp  Lys Asp Ser Leu Asn  Glu Ala Trp Ala Asp  Leu Leu Glu
    1775                 1780                 1785

ctg ctg  gac aca aga ggt cag  gtg ctg gct gct gct  tat gag ctg      5409
Leu Leu  Asp Thr Arg Gly Gln  Val Leu Ala Ala Ala  Tyr Glu Leu
    1790                 1795                 1800

cag cgc  ttc ctg cat ggg gcc  cgc caa gcc ctg gca  cgg gtg cag      5454
Gln Arg  Phe Leu His Gly Ala  Arg Gln Ala Leu Ala  Arg Val Gln
    1805                 1810                 1815

cac aag  cag cag cag ctt cca  gat ggg acg ggc cgc  gac ctc aat      5499
His Lys  Gln Gln Gln Leu Pro  Asp Gly Thr Gly Arg  Asp Leu Asn
    1820                 1825                 1830

gct gct  gag gcc ctg cag cgc  cgg cac tgc gcc tat  gag cac gac      5544
Ala Ala  Glu Ala Leu Gln Arg  Arg His Cys Ala Tyr  Glu His Asp
    1835                 1840                 1845

atc caa  gcc ctc agc act cag  gtc cag cag gtt cag  gac gat ggc      5589
Ile Gln  Ala Leu Ser Thr Gln  Val Gln Gln Val Gln  Asp Asp Gly
    1850                 1855                 1860

ctc agg  cta caa aag gcc tat  gct gga gac aag gct  gag gaa att      5634
Leu Arg  Leu Gln Lys Ala Tyr  Ala Gly Asp Lys Ala  Glu Glu Ile
    1865                 1870                 1875

ggc cgt  cac atg cag gca gtg  gct gag gcg tgg gcc  cag ctc cag      5679
Gly Arg  His Met Gln Ala Val  Ala Glu Ala Trp Ala  Gln Leu Gln
    1880                 1885                 1890

gga agt  tct gct gcc cgt cgc  cag ctg tta ctg gat  acc aca gac      5724
Gly Ser  Ser Ala Ala Arg Arg  Gln Leu Leu Leu Asp  Thr Thr Asp
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    1895                1900                1905

aaa ttc  cga ttc ttc aag gct  gtc cgg gag ttg atg  ctg tgg atg      5769
Lys Phe  Arg Phe Phe Lys Ala  Val Arg Glu Leu Met  Leu Trp Met
    1910                1915                1920

gat ggg  att aac ctg cag atg  gat gcc cag gag agg  ccc cgg gat      5814
Asp Gly  Ile Asn Leu Gln Met  Asp Ala Gln Glu Arg  Pro Arg Asp
    1925                1930                1935

gtg tcc  tct gca gat tta gtc  atc aaa aac caa caa  ggc atc aaa      5859
Val Ser  Ser Ala Asp Leu Val  Ile Lys Asn Gln Gln  Gly Ile Lys
    1940                1945                1950

gca gag  ata gag gca aga gct  gac agg ttc tcc gcc  tgc att gac      5904
Ala Glu  Ile Glu Ala Arg Ala  Asp Arg Phe Ser Ala  Cys Ile Asp
    1955                1960                1965

atg ggg  caa gag ctg ctg gcc  cgg aac cac tat gcc  gct gag gag      5949
Met Gly  Gln Glu Leu Leu Ala  Arg Asn His Tyr Ala  Ala Glu Glu
    1970                1975                1980

atc tca  gag aag ctg tct cag  cta cag tcc cgg cgc  cag gag aca      5994
Ile Ser  Glu Lys Leu Ser Gln  Leu Gln Ser Arg Arg  Gln Glu Thr
    1985                1990                1995

gct gaa  aag tgg cag gag aag  atg gac tgg cta cag  ctt gtt ttg      6039
Ala Glu  Lys Trp Gln Glu Lys  Met Asp Trp Leu Gln  Leu Val Leu
    2000                2005                2010

gag gtg  ctt gtg ttt ggg aga  gat gca ggc atg gca  gag gcc tgg      6084
Glu Val  Leu Val Phe Gly Arg  Asp Ala Gly Met Ala  Glu Ala Trp
    2015                2020                2025

cta tgc  agt cag gag cca ttg  gtg cga agt gca gaa  ctg ggt tgc      6129
Leu Cys  Ser Gln Glu Pro Leu  Val Arg Ser Ala Glu  Leu Gly Cys
    2030                2035                2040

act gtg  gat gaa gta gag agc  ctc atc aag cgg cat  gaa gcc ttc      6174
Thr Val  Asp Glu Val Glu Ser  Leu Ile Lys Arg His  Glu Ala Phe
    2045                2050                2055

cag aag  tca gca gtg gcc tgg  gag gag cgt ttc agt  gcc ctg gag      6219
Gln Lys  Ser Ala Val Ala Trp  Glu Glu Arg Phe Ser  Ala Leu Glu
    2060                2065                2070

aag ctc  act gcg ctg gaa gag  cgg gag aat gag cag  aaa agg aag      6264
Lys Leu  Thr Ala Leu Glu Glu  Arg Glu Asn Glu Gln  Lys Arg Lys
    2075                2080                2085

agg gag  gag gag gaa cga agg  aaa cag ccc cct act  tca gag ccc      6309
Arg Glu  Glu Glu Glu Arg Arg  Lys Gln Pro Pro Thr  Ser Glu Pro
    2090                2095                2100

atg gct  agt caa ccg gaa ggg  agt ctg gta gat ggc  cag aga gtt      6354
Met Ala  Ser Gln Pro Glu Gly  Ser Leu Val Asp Gly  Gln Arg Val
    2105                2110                2115

ctt gac  act gcc tgg gat ggg  acc cag tca aaa ttg  cca cca tcc      6399
Leu Asp  Thr Ala Trp Asp Gly  Thr Gln Ser Lys Leu  Pro Pro Ser
    2120                2125                2130

aca caa  gca ccc agc att aat  ggg gtc tgc acg gac  acg gag tcc      6444
Thr Gln  Ala Pro Ser Ile Asn  Gly Val Cys Thr Asp  Thr Glu Ser
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Ser Gln  Pro Leu Leu Glu Gln  Gln Arg Leu Glu Gln  Ser Asn Val
    2150                 2155                 2160

cca gaa  ggg cct gga tct ggc  aca gga gac gag tcc  agc ggg ccc      6534
Pro Glu  Gly Pro Gly Ser Gly  Thr Gly Asp Glu Ser  Ser Gly Pro
    2165                 2170                 2175

cgg gga  gag agg cag acc ctg  ccc cgg ggc cct gct  ccg tct cca      6579
Arg Gly  Glu Arg Gln Thr Leu  Pro Arg Gly Pro Ala  Pro Ser Pro
    2180                 2185                 2190

atg ccc  cag agc aga tcg tct  gag tca gct cat gtt  gcc acc ctg      6624
Met Pro  Gln Ser Arg Ser Ser  Glu Ser Ala His Val  Ala Thr Leu
    2195                 2200                 2205

ccc gca  cga ggt gct gag ctc  tct gct cag gaa cag  atg gaa ggg      6669
Pro Ala  Arg Gly Ala Glu Leu  Ser Ala Gln Glu Gln  Met Glu Gly
    2210                 2215                 2220

acg ctg  tgc cgc aaa cag gag  atg gaa gcc ttc aat  aag aaa gct      6714
Thr Leu  Cys Arg Lys Gln Glu  Met Glu Ala Phe Asn  Lys Lys Ala
    2225                 2230                 2235

gcc aac  agg tcc tgg cag aat  gtg tac tgt gta ctt  cgg cgt gga      6759
Ala Asn  Arg Ser Trp Gln Asn  Val Tyr Cys Val Leu  Arg Arg Gly
    2240                 2245                 2250

agc ctc  ggc ttt tac aag gat  gcc agg gca gct agt  gca gga gtg      6804
Ser Leu  Gly Phe Tyr Lys Asp  Ala Arg Ala Ala Ser  Ala Gly Val
    2255                 2260                 2265

cca tac  cat gga gaa gtg cct  gtc agt ctg gcc agg  gcc cag ggc      6849
Pro Tyr  His Gly Glu Val Pro  Val Ser Leu Ala Arg  Ala Gln Gly
    2270                 2275                 2280

agt gtg  gcc ttt gat tat cgg  aaa cgc aaa cat gtc  ttc aag ctg      6894
Ser Val  Ala Phe Asp Tyr Arg  Lys Arg Lys His Val  Phe Lys Leu
    2285                 2290                 2295

ggc ttg  cag gat ggg aaa gag  tat cta ttc cag gcc  aag gat gag      6939
Gly Leu  Gln Asp Gly Lys Glu  Tyr Leu Phe Gln Ala  Lys Asp Glu
    2300                 2305                 2310

gca gag  atg agc tca tgg ctg  aga gtg gtg aat gca  gcc att gcc      6984
Ala Glu  Met Ser Ser Trp Leu  Arg Val Val Asn Ala  Ala Ile Ala
    2315                 2320                 2325

act gcg  tcc tcg gcc tct gga  gag cca gaa gag cca  gtg gtg ccc      7029
Thr Ala  Ser Ser Ala Ser Gly  Glu Pro Glu Glu Pro  Val Val Pro
    2330                 2335                 2340

agt gcc  agc cgg ggt ctg acc  agg gcc atg acc atg  ccc cca gtg      7074
Ser Ala  Ser Arg Gly Leu Thr  Arg Ala Met Thr Met  Pro Pro Val
    2345                 2350                 2355

tca cag  cct gag ggc tcc atc  gtg ctt cgc agc aag  gat ggc aga      7119
Ser Gln  Pro Glu Gly Ser Ile  Val Leu Arg Ser Lys  Asp Gly Arg
    2360                 2365                 2370

gaa aga  gag cga gaa aaa cga  ttc agc ttc ttt aag  aag aac aag      7164
Glu Arg  Glu Arg Glu Lys Arg  Phe Ser Phe Phe Lys  Lys Asn Lys
    2375                 2380                 2385
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gtcgacagag accacc 7240

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<213> Rattus

<400> 2

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Lys Ala Leu Ala Asp Glu Arg Glu Ala Val Gln Lys Lys Thr Phe Thr
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Lys Trp Val Asn Ser His Leu Ala Arg Val Thr Cys Arg Val Gly Asp
65 70 75 80

Leu Tyr Ser Asp Leu Arg Asp Gly Arg Asn Leu Leu Arg Leu Leu Glu
85 90 95

Val Leu Ser Gly Glu Thr Leu Pro Lys Pro Thr Lys Gly Arg Met Arg
100 105 110

Ile His Cys Leu Glu Asn Val Asp Lys Ala Leu Gln Phe Leu Lys Glu
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Gln Lys Val His Leu Glu Asn Met Gly Ser His Asp Ile Val Asp Gly
130 135 140

Asn His Arg Leu Thr Leu Gly Leu Val Trp Thr Ile Ile Leu Arg Phe
145 150 155 160

Gln Ile Gln Asp Ile Ser Val Glu Thr Glu Asp Asn Lys Glu Lys Lys
165 170 175

Ser Ala Lys Asp Ala Leu Leu Leu Trp Cys Gln Met Lys Thr Ala Gly
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Tyr Pro Asn Val Asn Val His Asn Phe Thr Thr Ser Trp Arg Asp Gly
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Leu Ala Phe Asn Ala Ile Val His Lys His Arg Pro Asp Leu Leu Asp

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Phe Glu Ser Leu Lys Lys Cys Asn Ala His Tyr Asn Leu Gln Asn Ala
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Phe Asn Leu Ala Glu Lys Glu Leu Gly Leu Thr Lys Leu Leu Asp Pro
                245                 250                 255

Glu Asp Val Asn Val Asp Gln Pro Asp Glu Lys Ser Ile Ile Thr Tyr
            260                 265                 270

Val Ala Thr Tyr Tyr His Tyr Phe Ser Lys Met Lys Ala Leu Ala Val
        275                 280                 285

Glu Gly Lys Arg Ile Gly Lys Val Leu Asp His Ala Met Glu Ala Glu
    290                 295                 300

His Leu Val Glu Lys Tyr Glu Ser Leu Ala Ser Glu Leu Leu Gln Trp
305                 310                 315                 320

Ile Glu Gln Thr Ile Gly Thr Phe Asn Asp Arg Gln Leu Ala Asn Ser
                325                 330                 335

Leu Ser Gly Val Gln Asn Gln Leu Gln Ser Phe Asn Ser Tyr Arg Thr
            340                 345                 350

Val Glu Lys Pro Pro Lys Phe Thr Glu Lys Gly Asn Leu Glu Val Leu
        355                 360                 365

Leu Phe Thr Ile Gln Ser Lys Leu Arg Ala Asn Asn Gln Lys Val Tyr
    370                 375                 380

Thr Pro Arg Glu Gly Arg Leu Ile Ser Asp Ile Asn Lys Ala Trp Glu
385                 390                 395                 400

Arg Leu Glu Lys Ala Glu His Glu Arg Glu Leu Ala Leu Arg Thr Glu
                405                 410                 415

Leu Ile Arg Gln Glu Lys Leu Glu Gln Leu Ala Ala Arg Phe Asp Arg
            420                 425                 430

Lys Ala Ala Met Arg Glu Thr Trp Leu Ser Glu Asn Gln Arg Leu Val
        435                 440                 445

Ser Gln Asp Asn Phe Gly Leu Glu Leu Ala Ala Val Glu Ala Ala Val
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Arg Lys His Glu Ala Ile Glu Thr Asp Ile Val Ala Tyr Ser Gly Arg
465                 470                 475                 480
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Val Gln Ala Val Asp Ala Val Ala Ala Glu Leu Ala Ala Glu His Tyr
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His Asp Ile Lys Arg Ile Ala Ala Arg Gln Asn Asn Val Ala Arg Leu
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Trp Asp Phe Leu Arg Glu Met Val Ala Ala Arg Arg Glu Arg Leu Leu
515 520 525

Leu Asn Leu Glu Leu Gln Lys Val Phe Gln Asp Leu Leu Tyr Leu Met
530 535 540

Asp Trp Met Ala Glu Met Lys Gly Arg Leu Gln Ser Gln Asp Leu Gly
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Lys His Leu Ala Gly Val Glu Asp Leu Leu Gln Leu His Glu Leu Val
565 570 575

Glu Ala Asp Ile Ala Val Gln Ala Glu Arg Val Arg Ala Val Ser Ala
580 585 590

Ser Ala Leu Arg Phe Cys Asp Pro Gly Lys Glu Tyr Arg Pro Cys Gly
595 600 605

Pro Gln Leu Val Ser Glu Arg Val Ala Thr Leu Glu Gln Ser Tyr Glu
610 615 620

Ala Leu Cys Glu Leu Ala Ala Thr Arg Arg Ala Arg Leu Glu Glu Ser
625 630 635 640

Arg Arg Leu Trp Arg Phe Leu Trp Glu Val Gly Glu Ala Glu Ala Trp
645 650 655

Val Arg Glu Gln Gln His Leu Leu Ala Ser Ala Glu Thr Gly Arg Asp
660 665 670

Leu Thr Gly Val Leu Arg Leu Leu Asn Lys His Thr Ala Leu Arg Gly
675 680 685

Glu Met Ser Gly Arg Leu Gly Pro Leu Lys Leu Thr Leu Glu Gln Gly
690 695 700

Gln Gln Leu Val Ala Glu Gly His Pro Gly Ala Asn Gln Ala Ser Thr
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Arg Ala Ala Glu Leu Gln Ala Gln Trp Glu Arg Leu Glu Ala Leu Ala
725 730 735

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Leu Val Ser Ser Pro Glu Val Gly His Asp Glu Phe Ser Thr Gln Ala
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Leu Ala Arg Gln His Arg Ala Leu Glu Glu Glu Ile Arg Ala His Arg
785 790 795 800

Pro Thr Leu Asp Ala Leu Arg Glu Gln Ala Ala Ala Leu Pro Pro Ala
805 810 815

Leu Ser His Thr Pro Glu Val Gln Gly Arg Val Pro Thr Leu Glu Gln
820 825 830

His Tyr Glu Glu Leu Gln Ala Arg Ala Gly Glu Arg Ala Arg Ala Leu
835 840 845

Glu Ala Ala Leu Ala Phe Tyr Thr Met Leu Ser Glu Ala Gly Ala Cys
850 855 860

Gly Leu Trp Val Glu Glu Lys Glu Gln Trp Leu Asn Gly Leu Ala Leu
865 870 875 880

Pro Glu Arg Leu Glu Asp Pro Glu Val Val Gln Gln Arg Phe Glu Thr
885 890 895

Leu Glu Pro Glu Met Asn Ala Leu Ala Ala Arg Ile Thr Ala Val Ser
900 905 910

Asp Ile Ala Glu Gln Leu Leu Lys Ala Ser Pro Pro Gly Lys Asp Arg
915 920 925

Ile Ile Gly Thr Gln Glu Gln Leu Asn Gln Arg Trp Gln Gln Phe Arg
930 935 940

Ser Leu Ala Gly Gly Lys Lys Ala Ala Leu Thr Ser Ala Leu Ser Ile
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Gln Asn Tyr His Leu Glu Cys Thr Glu Thr Gln Ala Trp Met Arg Glu
965 970 975

Lys Thr Lys Val Ile Glu Ser Thr Gln Asp Leu Gly Asn Asp Leu Ala
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Gly Val Leu Ala Leu Gln Arg Lys Leu Ala Gly Thr Glu Arg Asp Leu
995 1000 1005

Glu Ala Ile Ser Ala Arg Val Gly Glu Leu Thr Gln Glu Ala Asn
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Ala Leu Ala Ala Gly His Pro Ala Gln Ala Pro Ala Ile Asn Thr
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Arg Leu Gly Glu Val Gln Thr Gly Trp Glu Asp Leu Arg Ala Thr
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Phe Gln Gly Phe Leu Arg Asp Ala Arg Gln Ala Glu Gly Val Leu
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Ser Ser Gln Glu Tyr Val Leu Ser His Thr Glu Met Pro Gly Thr
1190 1195 1200

Leu Gln Ala Ala Asp Ala Ala Ile Lys Lys Leu Glu Asp Phe Met
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Ser Thr Met Asp Ala Asn Gly Glu Arg Ile Arg Gly Leu Leu Glu
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Ala Gly Arg Gln Leu Val Ser Lys Gly Asn Ile His Ala Glu Lys

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Glu Gln  Gln His Phe Leu Gln  Asp Cys Gln Glu Leu  Lys Leu Trp
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Ile Asp  Glu Lys Met Leu Thr  Ala Gln Asp Val Ser  Tyr Asp Glu
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Ala Arg  Asn Leu His Thr Lys  Trp Gln Lys His Gln  Ala Phe Met
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Ala Glu  Leu Ala Ala Asn Lys  Asp Trp Leu Asp Lys  Val Asp Lys
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Glu Gly  Arg Glu Leu Thr Leu  Glu Lys Pro Glu Leu  Lys Val Leu
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Val Ser  Glu Lys Leu Glu Asp  Leu His Arg Arg Trp  Asp Glu Leu
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Glu Thr  Thr Thr Gln Ala Lys  Ala Arg Ser Leu Phe  Asp Ala Asn
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Arg Ala  Glu Leu Phe Ala Gln  Ser Cys Ser Ala Leu  Glu Ser Trp
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Leu Glu  Ser Leu Gln Ala Gln  Leu His Ser Asp Asp  Tyr Gly Lys
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Asp Leu  Thr Ser Val Asn Ile  Leu Leu Lys Lys Gln  Gln Met Leu
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Glu Arg  Glu Met Ala Val Arg  Glu Lys Glu Val Glu  Ala Ile Gln
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Ala Gln  Ala Lys Ala Leu Ala  Gln Glu Asp Gln Ser  Ala Gly Glu
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Val Glu  Arg Thr Ser Arg Ala  Val Glu Glu Lys Phe  Arg Ala Leu
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Cys Gln  Pro Met Lys Asp Arg  Cys Arg Arg Leu Gln  Ala Ser Arg
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Gln Lys Glu Ile Gln Gly His Glu Pro Arg Ile Ala Asp Leu Lys
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Leu Arg Gly Lys Arg Leu Glu Glu Ala Leu Arg Ala Gln Gln Phe
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Tyr Arg Asp Ala Ala Glu Ala Glu Ala Trp Met Gly Glu Gln Glu
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Gln Ala Glu Val Lys Lys His Gln Val Leu Glu Gln Ala Leu Ala
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Asp Tyr Ala Gln Thr Ile Lys Gln Leu Ala Ala Ser Ser Gln Asp
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Met Ile Asp His Glu His Pro Glu Ser Thr Arg Leu Thr Ile Arg
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Gln Ala Gln Val Asp Lys Leu Tyr Ala Gly Leu Lys Glu Leu Ala
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Gly Glu Arg Arg Glu Arg Leu Gln Glu His Leu Arg Leu Cys Gln
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Leu Arg Arg Glu Leu Asp Asp Leu Glu Gln Trp Ile Gln Glu Arg
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1715 1720 1725

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Val Thr Met Leu Arg Asp Lys Phe Arg Glu Phe Ser Arg Asp Thr
1730 1735 1740

Ser Thr Ile Gly Gln Glu Arg Val Asp Ser Ala Asn Ala Leu Ala
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Asn Gly Leu Ile Ala Gly Gly His Ala Ala Trp Ala Thr Val Ala
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Glu Trp Lys Asp Ser Leu Asn Glu Ala Trp Ala Asp Leu Leu Glu
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Leu Leu Asp Thr Arg Gly Gln Val Leu Ala Ala Ala Tyr Glu Leu
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Gln Arg Phe Leu His Gly Ala Arg Gln Ala Leu Ala Arg Val Gln
1805 1810 1815

His Lys Gln Gln Gln Leu Pro Asp Gly Thr Gly Arg Asp Leu Asn
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Ala Ala Glu Ala Leu Gln Arg Arg His Cys Ala Tyr Glu His Asp
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Ile Gln Ala Leu Ser Thr Gln Val Gln Gln Val Gln Asp Asp Gly
1850 1855 1860

Leu Arg Leu Gln Lys Ala Tyr Ala Gly Asp Lys Ala Glu Glu Ile
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Gly Arg His Met Gln Ala Val Ala Glu Ala Trp Ala Gln Leu Gln
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Gly Ser Ser Ala Ala Arg Arg Gln Leu Leu Leu Asp Thr Thr Asp
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Lys Phe Arg Phe Phe Lys Ala Val Arg Glu Leu Met Leu Trp Met
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Asp Gly Ile Asn Leu Gln Met Asp Ala Gln Glu Arg Pro Arg Asp
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1940 1945 1950

Ala Glu Ile Glu Ala Arg Ala Asp Arg Phe Ser Ala Cys Ile Asp
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Leu Asp Thr Ala Trp Asp Gly Thr Gln Ser Lys Leu Pro Pro Ser
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Ser Gln Pro Leu Leu Glu Gln Gln Arg Leu Glu Gln Ser Asn Val
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Pro Glu Gly Pro Gly Ser Gly Thr Gly Asp Glu Ser Ser Gly Pro
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Arg Gly Glu Arg Gln Thr Leu Pro Arg Gly Pro Ala Pro Ser Pro
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Met Pro Gln Ser Arg Ser Ser Glu Ser Ala His Val Ala Thr Leu
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Pro Ala Arg Gly Ala Glu Leu Ser Ala Gln Glu Gln Met Glu Gly

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Ala Asn Arg Ser Trp Gln Asn Val Tyr Cys Val Leu Arg Arg Gly
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Ser Leu Gly Phe Tyr Lys Asp Ala Arg Ala Ala Ser Ala Gly Val
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Pro Tyr His Gly Glu Val Pro Val Ser Leu Ala Arg Ala Gln Gly
2270 2275 2280

Ser Val Ala Phe Asp Tyr Arg Lys Arg Lys His Val Phe Lys Leu
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Gly Leu Gln Asp Gly Lys Glu Tyr Leu Phe Gln Ala Lys Asp Glu
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Ala Glu Met Ser Ser Trp Leu Arg Val Val Asn Ala Ala Ile Ala
2315 2320 2325

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2345 2350 2355

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Val Ile Ile Gln Lys Asp Gln His Gly Phe Gly Phe Thr Val Ser Gly
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gat cgc att gta ctg gtg cag tcc gtg cgc cct gga ggc gca gcc atg      288
Asp Arg Ile Val Leu Val Gln Ser Val Arg Pro Gly Gly Ala Ala Met
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aaa gct ggt gtg aaa gag ggt gac cgg atc atc aaa gta aac ggc acc      336
Lys Ala Gly Val Lys Glu Gly Asp Arg Ile Ile Lys Val Asn Gly Thr
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atg gtg acc aat agc tca cac ctg gag gtg gta aag ctt atc aaa tct      384
Met Val Thr Asn Ser Ser His Leu Glu Val Val Lys Leu Ile Lys Ser
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ggc gcc tat gct gcg ctt acc ctc cta ggc tct tct cct ccc tcc gtc      432
Gly Ala Tyr Ala Ala Leu Thr Leu Leu Gly Ser Ser Pro Pro Ser Val
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ggc gtc tct ggg ctc cag cag aat cca tct gtg gca gga gtg ctc aga      480
Gly Val Ser Gly Leu Gln Gln Asn Pro Ser Val Ala Gly Val Leu Arg
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gtt aac ccc atc att cct cca cca cct ccc ccg cca ccc ttg cca cct      528
Val Asn Pro Ile Ile Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Pro
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cca cag cac att act gga ccc aaa cct ctt cag gat cct gaa gtc caa      576
Pro Gln His Ile Thr Gly Pro Lys Pro Leu Gln Asp Pro Glu Val Gln
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aag cac gcc act caa atc ctc tgg aat atg cta aga cag gag gag gaa      624
Lys His Ala Thr Gln Ile Leu Trp Asn Met Leu Arg Gln Glu Glu Glu
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Glu Leu Gln Asp Ile Leu Pro Pro Cys Gly Glu Thr Ser Gln Arg Thr
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Cys Glu Gly Arg Leu Ser Val Asp Ser Gln Glu Ala Asp Ser Gly Leu
225                 230                 235                 240

gat tct ggg aca gaa cgc ttt ccc tcc atc agt gag tca ttg atg aat      768
Asp Ser Gly Thr Glu Arg Phe Pro Ser Ile Ser Glu Ser Leu Met Asn
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cgg aac tca gta ttg tca gat cct gga cta gac agc cct caa acc tcc      816
Arg Asn Ser Val Leu Ser Asp Pro Gly Leu Asp Ser Pro Gln Thr Ser
            260                 265                 270

cct gta atc ctg gcc agg gtg gcc cag cac cac agg cga cag ggc tca      864
Pro Val Ile Leu Ala Arg Val Ala Gln His His Arg Arg Gln Gly Ser
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gat gca gcg ttg ctc ccg ctc aac cac cag ggt ata gat caa agc cca      912
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Asp Ala Ala Leu Leu Pro Leu Asn His Gln Gly Ile Asp Gln Ser Pro
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Lys Pro Leu Ile Ile Gly Pro Glu Glu Asp Tyr Asp Pro Gly Tyr Phe
305                 310                 315                 320

aac aat gag agt gac atc atc ttc caa gat ctt gaa aaa ctg aag tca     1008
Asn Asn Glu Ser Asp Ile Ile Phe Gln Asp Leu Glu Lys Leu Lys Ser
                325                 330                 335

cat cca gct tac ttg gta gtt ttt cta cgt tac atc ctc tct cag gca     1056
His Pro Ala Tyr Leu Val Val Phe Leu Arg Tyr Ile Leu Ser Gln Ala
            340                 345                 350

gac cct ggc ccc ctg ctt ttt tat ttg tgt tca gaa gtt tat caa cag     1104
Asp Pro Gly Pro Leu Leu Phe Tyr Leu Cys Ser Glu Val Tyr Gln Gln
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Thr Asn Pro Lys Asp Ser Arg Ser Leu Gly Lys Asp Ile Trp Asn Ile
    370                 375                 380

ttc ctg gag aaa aat gcg cct ctc aga gtg aag atc cct gag atg ttg     1200
Phe Leu Glu Lys Asn Ala Pro Leu Arg Val Lys Ile Pro Glu Met Leu
385                 390                 395                 400

cag gct gaa att gac cta cgc ctg cgg aac aat gag gac cct cgc aat     1248
Gln Ala Glu Ile Asp Leu Arg Leu Arg Asn Asn Glu Asp Pro Arg Asn
                405                 410                 415

gtg ctc tgt gaa gct cag gag gca gtc atg ctg gaa atc cag gag cag     1296
Val Leu Cys Glu Ala Gln Glu Ala Val Met Leu Glu Ile Gln Glu Gln
            420                 425                 430

atc aac gac tac aga tcc aag cgt act ctg ggc ctg ggc agc ctc tat     1344
Ile Asn Asp Tyr Arg Ser Lys Arg Thr Leu Gly Leu Gly Ser Leu Tyr
        435                 440                 445

ggt gaa aat gac ctg cta ggc ctg gat ggg gac cct ctt cga gaa cgc     1392
Gly Glu Asn Asp Leu Leu Gly Leu Asp Gly Asp Pro Leu Arg Glu Arg
    450                 455                 460

caa atg gct gag aag cag ctg gct gcc ctt gga gat atc ttg tcc aaa     1440
Gln Met Ala Glu Lys Gln Leu Ala Ala Leu Gly Asp Ile Leu Ser Lys
465                 470                 475                 480

tat gag gaa gat cgg agt gcc ccc atg gac ttt gct gtt aat acc ttt     1488
Tyr Glu Glu Asp Arg Ser Ala Pro Met Asp Phe Ala Val Asn Thr Phe
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atg agc cac gct ggg atc cgt ctt cgg gag tct cga tcc tcc tgc acg     1536
Met Ser His Ala Gly Ile Arg Leu Arg Glu Ser Arg Ser Ser Cys Thr
            500                 505                 510

gca gaa aag acc cag tct gcc cct gac aag gac aag tgg ctg ccc ttc     1584
Ala Glu Lys Thr Gln Ser Ala Pro Asp Lys Asp Lys Trp Leu Pro Phe
        515                 520                 525

ttc cct aag acc aag aag cag agc agc aat tcc aag aaa gaa aag gat     1632
Phe Pro Lys Thr Lys Lys Gln Ser Ser Asn Ser Lys Lys Glu Lys Asp
    530                 535                 540

gcc ttg gag gac aag aag cga aac ccc atc ctc aga tat att ggg aag     1680
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                565                 570                 575

att cag cac ttt gag aac agc cat cag tat gat gtc cca gag ccg ggg     1776
Ile Gln His Phe Glu Asn Ser His Gln Tyr Asp Val Pro Glu Pro Gly
            580                 585                 590

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Thr Gln Arg Leu Ser Thr Gly Ser Phe Pro Glu Asp Leu Leu Glu Ser
        595                 600                 605

gac agt tcg cgc tca gag att cga ctg ggc cgc tct ggg agc ctc aag     1872
Asp Ser Ser Arg Ser Glu Ile Arg Leu Gly Arg Ser Gly Ser Leu Lys
    610                 615                 620

ggc cgg gaa gag atg aag cga tcc cgg aaa gca gag aac gtg ccc cgg     1920
Gly Arg Glu Glu Met Lys Arg Ser Arg Lys Ala Glu Asn Val Pro Arg
625                 630                 635                 640

cct cga agt gac gtt gac atg gat gct gct gca gag gct gcc cgc ctt     1968
Pro Arg Ser Asp Val Asp Met Asp Ala Ala Ala Glu Ala Ala Arg Leu
                645                 650                 655

cac cag tca gcc tcg tcc tct gcc tcc agc ctc tcc acc agg tct ctt     2016
His Gln Ser Ala Ser Ser Ser Ala Ser Ser Leu Ser Thr Arg Ser Leu
            660                 665                 670

gag aac cca acc cct ccc ttc acc ccc aaa atg ggc cgc agg agc att     2064
Glu Asn Pro Thr Pro Pro Phe Thr Pro Lys Met Gly Arg Arg Ser Ile
        675                 680                 685

gag tcc ccc aat ctg ggg ttc tgt aca gac gtc atc ctt ccc cac ctc     2112
Glu Ser Pro Asn Leu Gly Phe Cys Thr Asp Val Ile Leu Pro His Leu
    690                 695                 700

ctg gag gat gat ctg ggc caa ttg tct gac ctg gag cca gag cca gag     2160
Leu Glu Asp Asp Leu Gly Gln Leu Ser Asp Leu Glu Pro Glu Pro Glu
705                 710                 715                 720

gtc caa aac tgg cag cat aca gta ggc aag gat gtg gtg gcc aac ctg     2208
Val Gln Asn Trp Gln His Thr Val Gly Lys Asp Val Val Ala Asn Leu
                725                 730                 735

acc cag agg gaa att gac cgg caa gag gtc atc aat gag ctt ttt gtg     2256
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            740                 745                 750

aca gaa gca tct cac ctg cgc aca ctc cga gtc ctg gac ctc atc ttc     2304
Thr Glu Ala Ser His Leu Arg Thr Leu Arg Val Leu Asp Leu Ile Phe
        755                 760                 765

tac cag cgc atg aga aag gag aac cta atg cct cgg gaa gag cta gcg     2352
Tyr Gln Arg Met Arg Lys Glu Asn Leu Met Pro Arg Glu Glu Leu Ala
    770                 775                 780

cgg ctc ttc cct aac ctg cct gag ctc ata gag att cac aat tcc tgg     2400
Arg Leu Phe Pro Asn Leu Pro Glu Leu Ile Glu Ile His Asn Ser Trp
785                 790                 795                 800

tgt gag gcc atg aag aag ctc cgg gag gag ggc ccc att atc aga gac     2448
Cys Glu Ala Met Lys Lys Leu Arg Glu Glu Gly Pro Ile Ile Arg Asp
                805                 810                 815
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ctc cag caa gta gct gca caa ttc tgt tcc tat cag tca gta gcc cta      2544
Leu Gln Gln Val Ala Ala Gln Phe Cys Ser Tyr Gln Ser Val Ala Leu
        835                 840                 845

gag cta atc agg act aag caa cgt aag gag agt cgg ttc cag ctc ttc      2592
Glu Leu Ile Arg Thr Lys Gln Arg Lys Glu Ser Arg Phe Gln Leu Phe
    850                 855                 860

atg cag gag gct gag agc cac cct cag tgc cgg cgt ctg cag ctc cga      2640
Met Gln Glu Ala Glu Ser His Pro Gln Cys Arg Arg Leu Gln Leu Arg
865                 870                 875                 880

gac ctc atc gtc tct gaa atg caa cgg ctc acc aag tac cca ctg ctg      2688
Asp Leu Ile Val Ser Glu Met Gln Arg Leu Thr Lys Tyr Pro Leu Leu
                885                 890                 895

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Leu Glu Asn Ile Ile Lys His Thr Glu Gly Gly Thr Ser Glu His Glu
            900                 905                 910

aag ctc tgc cgt gcc cgg gac cag tgc cgg gag att ctc aag ttt gtg      2784
Lys Leu Cys Arg Ala Arg Asp Gln Cys Arg Glu Ile Leu Lys Phe Val
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aat gaa gca gta aag cag aca gag aac cgc cac cgg cta gag ggg tac      2832
Asn Glu Ala Val Lys Gln Thr Glu Asn Arg His Arg Leu Glu Gly Tyr
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cag aaa cgc ctg gat gcc act gcc cta gag cgg gcc agc aac ccc ttg      2880
Gln Lys Arg Leu Asp Ala Thr Ala Leu Glu Arg Ala Ser Asn Pro Leu
945                 950                 955                 960

gca gca gag ttc aag agc ctg gat ctt aca aca agg aag atg atc cac      2928
Ala Ala Glu Phe Lys Ser Leu Asp Leu Thr Thr Arg Lys Met Ile His
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gag ggg cct ctg acc tgg agg atc agc aaa gac aag acc ctg gac ctc      2976
Glu Gly Pro Leu Thr Trp Arg Ile Ser Lys Asp Lys Thr Leu Asp Leu
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cag gtg ctt ctg ctt gag gac ctg  gtg gta ctg ctg cag  aga caa gag    3024
Gln Val Leu Leu Leu Glu Asp Leu  Val Val Leu Leu Gln  Arg Gln Glu
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gag cgg  ctg ctg cta aag tgc  cac agc aag aca gcc  gtg ggc tcc       3069
Glu Arg  Leu Leu Leu Lys Cys  His Ser Lys Thr Ala  Val Gly Ser
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tcc gac  agc aag cag acg ttc  agc cct gtg ctg aag  ctc aat gct       3114
Ser Asp  Ser Lys Gln Thr Phe  Ser Pro Val Leu Lys  Leu Asn Ala
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gtg ctc  atc cgc tcc gtg gct  aca gac aag cga gcc  ttc ttc atc       3159
Val Leu  Ile Arg Ser Val Ala  Thr Asp Lys Arg Ala  Phe Phe Ile
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atc tgc  acc tcc gag ctg ggc  cct ccc cag atc tat  gag ctg gtt       3204
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gag gcc  gtg cag aat gcc acc  aag cac cct gga gct  gcc cca atc      3294
Glu Ala  Val Gln Asn Ala Thr  Lys His Pro Gly Ala  Ala Pro Ile
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ccc atc  cat ccc tca cca cca  gga tcc cag gag ccg  gcc tac cag      3339
Pro Ile  His Pro Ser Pro Pro  Gly Ser Gln Glu Pro  Ala Tyr Gln
    1100                 1105                 1110

ggc tcc  acc tcc agc agg gta  gaa ata aat gac tca  gaa gta tat      3384
Gly Ser  Thr Ser Ser Arg Val  Glu Ile Asn Asp Ser  Glu Val Tyr
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cac act  gaa aaa gaa ccc aag  aag cta cct gaa ggc  ccc ggg cct      3429
His Thr  Glu Lys Glu Pro Lys  Lys Leu Pro Glu Gly  Pro Gly Pro
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gag cag  aga gtt caa gac aag  cag ctg ata gca caa  ggg gag cct      3474
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Val Gln  Glu Glu Asp Glu Glu  Glu Leu Arg Thr Leu  Pro Arg Ala
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ccc ccc  tcc ctg gat gga gaa  aac aga ggc atc agg  aca agg gac      3564
Pro Pro  Ser Leu Asp Gly Glu  Asn Arg Gly Ile Arg  Thr Arg Asp
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cct gtc  ctt ctg gcc ctc aca  ggc cct ctg ctc atg  gag gga ctt      3609
Pro Val  Leu Leu Ala Leu Thr  Gly Pro Leu Leu Met  Glu Gly Leu
    1190                 1195                 1200

gct gat  gct gcc ctg gaa gat  gtg gag aac ttg cgt  cac ctg atc      3654
Ala Asp  Ala Ala Leu Glu Asp  Val Glu Asn Leu Arg  His Leu Ile
    1205                 1210                 1215

ctg tgg  agc ctg ctg cct ggt  cac act gtg aag act  cag gct gct      3699
Leu Trp  Ser Leu Leu Pro Gly  His Thr Val Lys Thr  Gln Ala Ala
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ggc gag  cct gag gat gac ctc  aca ccc acc cct tct  gtc gtg agc      3744
Gly Glu  Pro Glu Asp Asp Leu  Thr Pro Thr Pro Ser  Val Val Ser
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atc acc  tct cac ccc tgg gac  cca ggg tcc cca ggg  caa gct ccc      3789
Ile Thr  Ser His Pro Trp Asp  Pro Gly Ser Pro Gly  Gln Ala Pro
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acc ata  agt gac agc acc cga  ctt gcg agg cca gag  ggc agc cag      3834
Thr Ile  Ser Asp Ser Thr Arg  Leu Ala Arg Pro Glu  Gly Ser Gln
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cca gag  ggc gag gat gtt gct  gtc agt tct ctg gca  cac ctg ccg      3879
Pro Glu  Gly Glu Asp Val Ala  Val Ser Ser Leu Ala  His Leu Pro
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cca agg  acc aga agt tct ggc  gtc tgg gac tct cct  gag ctg gat      3924
Pro Arg  Thr Arg Ser Ser Gly  Val Trp Asp Ser Pro  Glu Leu Asp
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agg aat  ccg gct gca gag gct  gca agc aca gaa cca  gca gca agt      3969
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Tyr Lys  Val Val Arg Lys Val  Ser Leu Leu Pro Gly  Gly Gly Val
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ggt gca  gcc aag gtg gcg ggc  agc aat gct atc cct  gac agt ggc      4059
Gly Ala  Ala Lys Val Ala Gly  Ser Asn Ala Ile Pro  Asp Ser Gly
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cag tca  gaa tct gag cta tct  gaa gtg gaa ggc gga  gca cag gct      4104
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acg ggg  aac tgt ttc tat gtc  agc atg cca gca gga  cct ctg gac      4149
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Ser Ser  Thr Glu Pro Thr Gly  Thr Pro Pro Ser Pro  Ser Gln Cys
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Val Arg  Gly Gly Gln Cys Ser  Ser Leu Val Arg Arg  Asp Val Asp
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Val Ile  Phe His Thr Ile Glu  Gln Leu Thr Ile Lys  Leu His Arg
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ctg aag  gac atg gag ctg gcc  cac aga gag ctg ctc  aag tcc ctt      4374
Leu Lys  Asp Met Glu Leu Ala  His Arg Glu Leu Leu  Lys Ser Leu
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Gly Gly  Glu Ser Ser Gly Gly  Thr Thr Pro Val Gly  Ser Phe His
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Ser Cys  Pro Glu Glu Gly Ser  Asp Ile Ala Leu Glu  Asp Ser Ala
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Gln Pro Ser Asp Thr Ser Glu Thr Thr Ala Gly Leu Val Gln Arg Cys
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Val Ile Ile Gln Lys Asp Gln His Gly Phe Gly Phe Thr Val Ser Gly
65                  70                  75                  80

Asp Arg Ile Val Leu Val Gln Ser Val Arg Pro Gly Gly Ala Ala Met
                85                  90                  95

Lys Ala Gly Val Lys Glu Gly Asp Arg Ile Ile Lys Val Asn Gly Thr
            100                 105                 110

Met Val Thr Asn Ser Ser His Leu Glu Val Val Lys Leu Ile Lys Ser
        115                 120                 125

Gly Ala Tyr Ala Ala Leu Thr Leu Leu Gly Ser Ser Pro Pro Ser Val
    130                 135                 140

Gly Val Ser Gly Leu Gln Gln Asn Pro Ser Val Ala Gly Val Leu Arg
145                 150                 155                 160

Val Asn Pro Ile Ile Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Pro
                165                 170                 175

Pro Gln His Ile Thr Gly Pro Lys Pro Leu Gln Asp Pro Glu Val Gln
            180                 185                 190

Lys His Ala Thr Gln Ile Leu Trp Asn Met Leu Arg Gln Glu Glu Glu
        195                 200                 205

Glu Leu Gln Asp Ile Leu Pro Pro Cys Gly Glu Thr Ser Gln Arg Thr
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Cys Glu Gly Arg Leu Ser Val Asp Ser Gln Glu Ala Asp Ser Gly Leu
225                 230                 235                 240

Asp Ser Gly Thr Glu Arg Phe Pro Ser Ile Ser Glu Ser Leu Met Asn
                245                 250                 255
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Arg Asn Ser Val Leu Ser Asp Pro Gly Leu Asp Ser Pro Gln Thr Ser
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Pro Val Ile Leu Ala Arg Val Ala Gln His His Arg Arg Gln Gly Ser
275 280 285

Asp Ala Ala Leu Leu Pro Leu Asn His Gln Gly Ile Asp Gln Ser Pro
290 295 300

Lys Pro Leu Ile Ile Gly Pro Glu Glu Asp Tyr Asp Pro Gly Tyr Phe
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Asn Asn Glu Ser Asp Ile Ile Phe Gln Asp Leu Glu Lys Leu Lys Ser
325 330 335

His Pro Ala Tyr Leu Val Val Phe Leu Arg Tyr Ile Leu Ser Gln Ala
340 345 350

Asp Pro Gly Pro Leu Leu Phe Tyr Leu Cys Ser Glu Val Tyr Gln Gln
355 360 365

Thr Asn Pro Lys Asp Ser Arg Ser Leu Gly Lys Asp Ile Trp Asn Ile
370 375 380

Phe Leu Glu Lys Asn Ala Pro Leu Arg Val Lys Ile Pro Glu Met Leu
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Gln Ala Glu Ile Asp Leu Arg Leu Arg Asn Asn Glu Asp Pro Arg Asn
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Val Leu Cys Glu Ala Gln Glu Ala Val Met Leu Glu Ile Gln Glu Gln
420 425 430

Ile Asn Asp Tyr Arg Ser Lys Arg Thr Leu Gly Leu Gly Ser Leu Tyr
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Gly Glu Asn Asp Leu Leu Gly Leu Asp Gly Asp Pro Leu Arg Glu Arg
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Gln Met Ala Glu Lys Gln Leu Ala Ala Leu Gly Asp Ile Leu Ser Lys
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Tyr Glu Glu Asp Arg Ser Ala Pro Met Asp Phe Ala Val Asn Thr Phe
485 490 495

Met Ser His Ala Gly Ile Arg Leu Arg Glu Ser Arg Ser Ser Cys Thr
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Phe Pro Lys Thr Lys Lys Gln Ser Ser Asn Ser Lys Lys Glu Lys Asp
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Ala Leu Glu Asp Lys Lys Arg Asn Pro Ile Leu Arg Tyr Ile Gly Lys
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Pro Lys Ser Ser Ser Gln Ser Ile Lys Pro Gly Asn Val Arg Asn Ile
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Ile Gln His Phe Glu Asn Ser His Gln Tyr Asp Val Pro Glu Pro Gly
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Thr Gln Arg Leu Ser Thr Gly Ser Phe Pro Glu Asp Leu Leu Glu Ser
595 600 605

Asp Ser Ser Arg Ser Glu Ile Arg Leu Gly Arg Ser Gly Ser Leu Lys
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Gly Arg Glu Glu Met Lys Arg Ser Arg Lys Ala Glu Asn Val Pro Arg
625 630 635 640

Pro Arg Ser Asp Val Asp Met Asp Ala Ala Ala Glu Ala Ala Arg Leu
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His Gln Ser Ala Ser Ser Ser Ala Ser Ser Leu Ser Thr Arg Ser Leu
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Glu Asn Pro Thr Pro Pro Phe Thr Pro Lys Met Gly Arg Arg Ser Ile
675 680 685

Glu Ser Pro Asn Leu Gly Phe Cys Thr Asp Val Ile Leu Pro His Leu
690 695 700

Leu Glu Asp Asp Leu Gly Gln Leu Ser Asp Leu Glu Pro Glu Pro Glu
705 710 715 720

Val Gln Asn Trp Gln His Thr Val Gly Lys Asp Val Val Ala Asn Leu
725 730 735

Thr Gln Arg Glu Ile Asp Arg Gln Glu Val Ile Asn Glu Leu Phe Val
740 745 750

Thr Glu Ala Ser His Leu Arg Thr Leu Arg Val Leu Asp Leu Ile Phe
755 760 765

Tyr Gln Arg Met Arg Lys Glu Asn Leu Met Pro Arg Glu Glu Leu Ala

Substitute Sheet (Rule 26)

770 775 780

Arg Leu Phe Pro Asn Leu Pro Glu Leu Ile Glu Ile His Asn Ser Trp
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Cys Glu Ala Met Lys Lys Leu Arg Glu Glu Gly Pro Ile Ile Arg Asp
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Ile Ser Asp Pro Met Leu Ala Arg Phe Asp Gly Pro Ala Arg Glu Glu
820 825 830

Leu Gln Gln Val Ala Ala Gln Phe Cys Ser Tyr Gln Ser Val Ala Leu
835 840 845

Glu Leu Ile Arg Thr Lys Gln Arg Lys Glu Ser Arg Phe Gln Leu Phe
850 855 860

Met Gln Glu Ala Glu Ser His Pro Gln Cys Arg Arg Leu Gln Leu Arg
865 870 875 880

Asp Leu Ile Val Ser Glu Met Gln Arg Leu Thr Lys Tyr Pro Leu Leu
885 890 895

Leu Glu Asn Ile Ile Lys His Thr Glu Gly Gly Thr Ser Glu His Glu
900 905 910

Lys Leu Cys Arg Ala Arg Asp Gln Cys Arg Glu Ile Leu Lys Phe Val
915 920 925

Asn Glu Ala Val Lys Gln Thr Glu Asn Arg His Arg Leu Glu Gly Tyr
930 935 940

Gln Lys Arg Leu Asp Ala Thr Ala Leu Glu Arg Ala Ser Asn Pro Leu
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Ala Ala Glu Phe Lys Ser Leu Asp Leu Thr Thr Arg Lys Met Ile His
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Gln Val Leu Leu Leu Glu Asp Leu Val Val Leu Leu Gln Arg Gln Glu
995 1000 1005

Glu Arg Leu Leu Leu Lys Cys His Ser Lys Thr Ala Val Gly Ser
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Ser Asp Ser Lys Gln Thr Phe Ser Pro Val Leu Lys Leu Asn Ala
1025 1030 1035

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Val Leu Ile Arg Ser Val Ala Thr Asp Lys Arg Ala Phe Phe Ile
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Ile Cys Thr Ser Glu Leu Gly Pro Pro Gln Ile Tyr Glu Leu Val
1055 1060 1065

Ala Leu Thr Ser Ser Asp Lys Asn Ile Trp Met Glu Leu Leu Glu
1070 1075 1080

Glu Ala Val Gln Asn Ala Thr Lys His Pro Gly Ala Ala Pro Ile
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Pro Ile His Pro Ser Pro Pro Gly Ser Gln Glu Pro Ala Tyr Gln
1100 1105 1110

Gly Ser Thr Ser Ser Arg Val Glu Ile Asn Asp Ser Glu Val Tyr
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His Thr Glu Lys Glu Pro Lys Lys Leu Pro Glu Gly Pro Gly Pro
1130 1135 1140

Glu Gln Arg Val Gln Asp Lys Gln Leu Ile Ala Gln Gly Glu Pro
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Val Gln Glu Glu Asp Glu Glu Glu Leu Arg Thr Leu Pro Arg Ala
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Pro Pro Ser Leu Asp Gly Glu Asn Arg Gly Ile Arg Thr Arg Asp
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Pro Val Leu Leu Ala Leu Thr Gly Pro Leu Leu Met Glu Gly Leu
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Ala Asp Ala Ala Leu Glu Asp Val Glu Asn Leu Arg His Leu Ile
1205 1210 1215

Leu Trp Ser Leu Leu Pro Gly His Thr Val Lys Thr Gln Ala Ala
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Gly Glu Pro Glu Asp Asp Leu Thr Pro Thr Pro Ser Val Val Ser
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Ile Thr Ser His Pro Trp Asp Pro Gly Ser Pro Gly Gln Ala Pro
1250 1255 1260

Thr Ile Ser Asp Ser Thr Arg Leu Ala Arg Pro Glu Gly Ser Gln
1265 1270 1275

Pro Glu Gly Glu Asp Val Ala Val Ser Ser Leu Ala His Leu Pro
1280 1285 1290

Pro Arg Thr Arg Ser Ser Gly Val Trp Asp Ser Pro Glu Leu Asp
1295 1300 1305

Arg Asn Pro Ala Ala Glu Ala Ala Ser Thr Glu Pro Ala Ala Ser
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Tyr Lys Val Val Arg Lys Val Ser Leu Leu Pro Gly Gly Gly Val
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Gly Ala Ala Lys Val Ala Gly Ser Asn Ala Ile Pro Asp Ser Gly
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Gln Ser Glu Ser Glu Leu Ser Glu Val Glu Gly Gly Ala Gln Ala
1355 1360 1365

Thr Gly Asn Cys Phe Tyr Val Ser Met Pro Ala Gly Pro Leu Asp
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Ser Ser Thr Glu Pro Thr Gly Thr Pro Pro Ser Pro Ser Gln Cys
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His Ser Leu Pro Ala Trp Pro Thr Glu Pro Gln Pro Tyr Arg Gly
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Val Arg Gly Gly Gln Cys Ser Ser Leu Val Arg Arg Asp Val Asp
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Val Ile Phe His Thr Ile Glu Gln Leu Thr Ile Lys Leu His Arg
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Leu Lys Asp Met Glu Leu Ala His Arg Glu Leu Leu Lys Ser Leu
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Gly Gly Glu Ser Ser Gly Gly Thr Thr Pro Val Gly Ser Phe His
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Thr Glu Ala Ala Arg Trp Thr Asp Tyr Ser Leu Ser Pro Pro Ala
1475 1480 1485

Lys Glu Ala Leu Ala Ser Asp Ser Gln Asn Gly Gln Glu Gln Gly
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Application No. 09/915,364

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ggc tct gat cgt ttc gca cgg ccg gac ttc agg gat ata tcc aaa tgg       96
Gly Ser Asp Arg Phe Ala Arg Pro Asp Phe Arg Asp Ile Ser Lys Trp
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aac aac cgt gta gtg agc aat ctg ctc tat tac cag acc aac tac ctg      144
Asn Asn Arg Val Val Ser Asn Leu Leu Tyr Tyr Gln Thr Asn Tyr Leu
        35                  40                  45

gtg gtg gct gcc atg atg att tca gtc gtt ggg ttt ctg agc ccc ttc      192
Val Val Ala Ala Met Met Ile Ser Val Val Gly Phe Leu Ser Pro Phe
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aac atg atc ctt gga gga atc att gtg gtg ctg gtg ttc acg ggg ttt      240
Asn Met Ile Leu Gly Gly Ile Ile Val Val Leu Val Phe Thr Gly Phe
65                  70                  75                  80

gtg tgg gca gca cac aat aaa gac atc ctc cgc cgg atg aag aag cag      288
Val Trp Ala Ala His Asn Lys Asp Ile Leu Arg Arg Met Lys Lys Gln
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Tyr Pro Thr Ala Phe Val Met Val Val Met Leu Ala Ser Tyr Phe Leu
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Ile Ser Met Phe Gly Gly Val Met Val Phe Val Phe Gly Ile Thr Phe
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ccc tta ttg ttg atg ttc atc cat gca tcc ctg aga ctt cga aac ctc      432
Pro Leu Leu Leu Met Phe Ile His Ala Ser Leu Arg Leu Arg Asn Leu
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aag aac aaa ctg gaa aat aaa atg gag gga ata ggc ttg aag aaa acg      480
Lys Asn Lys Leu Glu Asn Lys Met Glu Gly Ile Gly Leu Lys Lys Thr
145                 150                 155                 160

ccg atg ggc atc atc ctg gat gcc ttg gaa cag cag gaa gac agc atc      528
Pro Met Gly Ile Ile Leu Asp Ala Leu Glu Gln Gln Glu Asp Ser Ile
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Asn Met Ile Leu Gly Gly Ile Ile Val Val Leu Val Phe Thr Gly Phe
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Val Trp Ala Ala His Asn Lys Asp Ile Leu Arg Arg Met Lys Lys Gln
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Tyr Pro Thr Ala Phe Val Met Val Val Met Leu Ala Ser Tyr Phe Leu
100 105 110

Ile Ser Met Phe Gly Gly Val Met Val Phe Val Phe Gly Ile Thr Phe
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Pro Leu Leu Leu Met Phe Ile His Ala Ser Leu Arg Leu Arg Asn Leu
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Lys Asn Lys Leu Glu Asn Lys Met Glu Gly Ile Gly Leu Lys Lys Thr
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accattaacc tacctctaca tccccctggc gccgttccag ggccaacgcc acattccctg 180

ctgggcacgc aatggccgca ccccctcccg ctacagaagg ctcttttggt acacgcagtc 240

cgaggtcgcc atg gat cgg atg aag aag atc aaa cgg cag ctg tca atg 289
Met Asp Arg Met Lys Lys Ile Lys Arg Gln Leu Ser Met
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Thr Leu Arg Gly Gly Arg Gly Ile Asp Lys Thr Asn Gly Val Pro Glu
15 20 25

cag ata ggc cta gat gag agt ggt ggt ggt ggt ggc atg acc ctt gga 385
Gln Ile Gly Leu Asp Glu Ser Gly Gly Gly Gly Gly Met Thr Leu Gly
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gaa gct ccc acc cgt gtt gcc cct ggg gaa ctt cgc tct att cgg ggc 433
Glu Ala Pro Thr Arg Val Ala Pro Gly Glu Leu Arg Ser Ile Arg Gly
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cca ctc agc tct gca cca g gtctacctgg gtttcccagt ctgctctagg 482
Pro Leu Ser Ser Ala Pro
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ggccatgtac acaaatggat acgatgaaga aatatattat attgggggaa agagagtgtt 542

cttgactcca aaggcctggc ctttccctca ctctgcacca g ag att gtg cat gaa 597
Glu Ile Val His Glu
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gac atg aag atg gga tct gat ggg gag agt gac cag gct tca gcc aca 645
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tcc tca gat gag gtg cag tct cca gtg aga gtg cgc atg cgc aac cac 693
Ser Ser Asp Glu Val Gln Ser Pro Val Arg Val Arg Met Arg Asn His
90 95 100

ccc cca cgc aag atc tcc act gag gat atc aac aaa tgc ctg tca cta 741
Pro Pro Arg Lys Ile Ser Thr Glu Asp Ile Asn Lys Cys Leu Ser Leu
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cca gct gac ata cgg ctg cct gag ggc tac ctt gag aag ctg acc ctc 789
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aat agc ccc atc ggt gat aag cct ctt agc cgg cgc ctc cgg cca gtc 837

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Ser Leu Ser Glu Ile Gly Phe Gly Lys Leu Glu Thr Tyr Ile Lys Leu
        155                 160                 165

gac aag ctg ggt gag ggt acc tat gcc act gtc tac aaa ggc aaa agc      933
Asp Lys Leu Gly Glu Gly Thr Tyr Ala Thr Val Tyr Lys Gly Lys Ser
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aag ctc aca gac aac ctt gta gca ctt aag gag atc aga ctg gaa cac      981
Lys Leu Thr Asp Asn Leu Val Ala Leu Lys Glu Ile Arg Leu Glu His
185                 190                 195                 200

gaa gaa ggg gca ccc tgc act gct atc cgg gaa gta tcc ctg ctt aag     1029
Glu Glu Gly Ala Pro Cys Thr Ala Ile Arg Glu Val Ser Leu Leu Lys
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gac ctc aag cat gcc aac atc gtc aca cta cat gac att atc cac aca     1077
Asp Leu Lys His Ala Asn Ile Val Thr Leu His Asp Ile Ile His Thr
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gag aag tcc ctc acc ctt gtc ttt gaa tac ttg gac aag gac ctg aag     1125
Glu Lys Ser Leu Thr Leu Val Phe Glu Tyr Leu Asp Lys Asp Leu Lys
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cag tac ctg gat gac tgt gga aat gtc atc aac atg cac aat gtg aaa     1173
Gln Tyr Leu Asp Asp Cys Gly Asn Val Ile Asn Met His Asn Val Lys
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ctg ttc ctg ttc cag ttg ctc cgt ggc ctg gcc tac tgc cac agg cag     1221
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265                 270                 275                 280

aag gtg cta cac cga gac ctc aag ccc cag aac cta ctc atc aac gag     1269
Lys Val Leu His Arg Asp Leu Lys Pro Gln Asn Leu Leu Ile Asn Glu
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agg gga gag ctc aaa ctg gca gac ttt ggc ctg gct tac gcc aag tca     1317
Arg Gly Glu Leu Lys Leu Ala Asp Phe Gly Leu Ala Tyr Ala Lys Ser
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Ile Pro Thr Lys Thr Tyr Ser Asn Glu Val Val Thr Leu Trp Tyr Arg
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ccc cct gac atc tta ctt ggg tcc aca gac tac tcc ggc caa att gac     1413
Pro Pro Asp Ile Leu Leu Gly Ser Thr Asp Tyr Ser Gly Gln Ile Asp
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atg tgg ggt gtt ggc tgc atc ttt tat gag atg gcc aca ggc cgg ccc     1461
Met Trp Gly Val Gly Cys Ile Phe Tyr Glu Met Ala Thr Gly Arg Pro
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ctc ttc cca ggc tcc aca gtg gaa gaa cag ctg cac ttc atc ttc cgc     1509
Leu Phe Pro Gly Ser Thr Val Glu Glu Gln Leu His Phe Ile Phe Arg
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att ttg gga acc cca act gag gac aca tgg cca ggt atc ctg tcc aat     1557
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gaa gag ttt aga aca tac aac tac ccc aag tac cga gcc gag gcc ctt     1605
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Lys Leu Leu Gln Phe Glu Gly Arg Asn Arg Ile Ser Ala Glu Asp Ala
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atg aaa cat cca ttc ttt ctc agc ttg ggg gag cgg atc cac aaa ctt     1749
Met Lys His Pro Phe Phe Leu Ser Leu Gly Glu Arg Ile His Lys Leu
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cct gac act act tcc ata ttt gca cta aag gag gta cag cta caa aag     1797
Pro Asp Thr Thr Ser Ile Phe Ala Leu Lys Glu Val Gln Leu Gln Lys
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Thr Arg Val Ala Pro Gly Glu Leu Arg Ser Ile Arg Gly Pro Leu Ser
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Ser Ala Pro Glu Ile Val His Glu Asp Met Lys Met Gly Ser Asp Gly
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Val Arg Val Arg Met Arg Asn His Pro Pro Arg Lys Ile Ser Thr Glu
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Asp Ile Asn Lys Cys Leu Ser Leu Pro Ala Asp Ile Arg Leu Pro Glu
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Gly Tyr Leu Glu Lys Leu Thr Leu Asn Ser Pro Ile Gly Asp Lys Pro
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Leu Ser Arg Arg Leu Arg Pro Val Ser Leu Ser Glu Ile Gly Phe Gly
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Lys Leu Glu Thr Tyr Ile Lys Leu Asp Lys Leu Gly Glu Gly Thr Tyr
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Ile Arg Glu Val Ser Leu Leu Lys Asp Leu Lys His Ala Asn Ile Val
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Thr Leu His Asp Ile Ile His Thr Glu Lys Ser Leu Thr Leu Val Phe
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Glu Tyr Leu Asp Lys Asp Leu Lys Gln Tyr Leu Asp Asp Cys Gly Asn
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Val Ile Asn Met His Asn Val Lys Leu Phe Leu Phe Gln Leu Leu Arg
260 265 270

Gly Leu Ala Tyr Cys His Arg Gln Lys Val Leu His Arg Asp Leu Lys
275 280 285

Pro Gln Asn Leu Leu Ile Asn Glu Arg Gly Glu Leu Lys Leu Ala Asp
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Phe Gly Leu Ala Tyr Ala Lys Ser Ile Pro Thr Lys Thr Tyr Ser Asn
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Glu Val Val Thr Leu Trp Tyr Arg Pro Pro Asp Ile Leu Leu Gly Ser
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Thr Asp Tyr Ser Gly Gln Ile Asp Met Trp Gly Val Gly Cys Ile Phe
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Tyr Glu Met Ala Thr Gly Arg Pro Leu Phe Pro Gly Ser Thr Val Glu
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Glu Gln Leu His Phe Ile Phe Arg Ile Leu Gly Thr Pro Thr Glu Asp
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Thr Trp Pro Gly Ile Leu Ser Asn Glu Glu Phe Arg Thr Tyr Asn Tyr
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Pro Lys Tyr Arg Ala Glu Ala Leu Leu Arg His Ala Pro Arg Leu Glu
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Cys Asp Gly Ala Asp Leu Leu Thr Lys Leu Leu Gln Phe Glu Gly Arg
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Asn Arg Ile Ser Ala Glu Asp Ala Met Lys His Pro Phe Phe Leu Ser
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Leu Gly Glu Arg Ile His Lys Leu Pro Asp Thr Thr Ser Ile Phe Ala
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Leu Lys Glu Val Gln Leu Gln Lys Glu Ala Asn Ile Arg Ser Thr Ser
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